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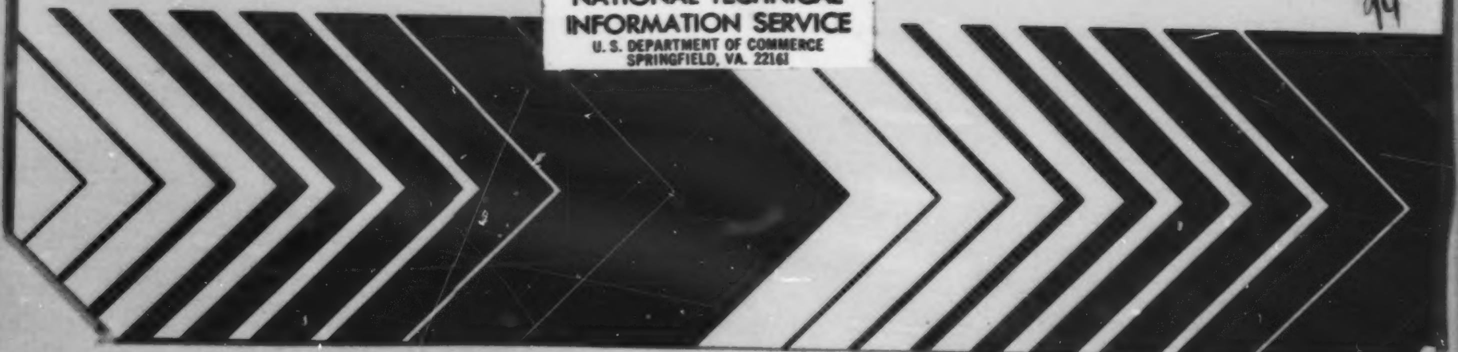

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This document is a review of the scientific literature on the biological and environmental effects of hexachlorocyclopentadiene. Inlcuded in the review are a general summary and a comprehensive discussion of the following topics as related to hexachlorocyclopentadiene and specific Hexachlorocyclopentadiene compounds: physical and chemical properties; occurrence; synthesis and use; analytical methodology; biological aspects in microorganisms, plants, wild and domestic animals, and humans; distribution mobility, and persistence in the environment; and an assessment of present and potential health and environmental hazards. More than 100 references are cited. The document also contains an evaluation of potential hazard resulting from hexachlorocyclopentadiene contamination in the enviornment and suggests current research needs.

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REVIEWS OF THE ENVIRONMENTAL EFFECTS OF POLLUTANTS:
XII. HEXACHLOROCYCLOPENTADIENE

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FOREWORD

A vast amount of published material is accumulating as numerous research investigations are conducted to develop a data base on the adverse effects of environmental pollution. As this information is amassed, it becomes continually more critical to focus on pertinent, well-designed studies. Research data must be summarized and interpreted in order to adequately evaluate the potential hazards of these substances to ecosystems and ultimately to public health.

The series of documents entitled "Reviews of the Enviornmental Effects of Pollutants" (REEPs) represents an extensive compilation of relevant research and forms an up-to-date compendium of the environmental effect data on selected pollutants.

The Review of the Environmental Effects of Hexachlorocyclopentadiene includes information on the chemical and physical properties of both compounds; pertinent analytical techniques; transport processes to the environment and subsequent distribution and deposition; impact on microorganisms, plants, and wildlife; toxicologic data in experimental animals including metabolism, toxicity, mutagenicity, teratogenicity and carcinogenicity; and an assessment of their health effects in man.

The REEPS are intended to serve various technical and administrative personnel within the Agency in the decision-making processes; i.e. in the development of criteria documents and environmental standards, and for other regulatory actions.*

* The breadth of these documents makes them a useful resource for public health personnel, environmental specialists, and control officers. Upon request these documents will be made available to any interested individuals or firms, both in and out of the Government. Depending on the supply, you can obtain the document directly by writing to:

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Jerry F. Stara

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Director
Environmental Criteria and
Assessment Office

PREFACE

Hexachlorocyclopentadiene ("hex") is one of those organic chemicals manufactured and used in substantial quantities within the chemical industry, but which are almost unknown outside the industry. Although it is an important intermediate in the manufacture of a number of organochlorine pesticides and flame retardants, it has essentially no end uses of its own. Thus, it is not surprising that its potential impact upon the environment has been studied very little, and that little is known about its environmental behavior, or its effects upon the biosphere or upon humans.

This REEP document is an attempt to gather together and recapitulate what is known about hex. It is painfully clear that there are large gaps in the information on its effects, particularly at chronic or sub-acute levels. More needs to be learned about hex to ensure that its use in manufacturing pesticides and flame retardants useful to man is safely conducted, so that environmental problems do not arise.

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ABSTRACT

The objective of this research program was to assemble in a publicly - available document information on the environmental effects of hexachlorocyclopentadiene (hex), a topic on which the published literature is still extremely sparse. A significant fraction of the information contained in this review consists of heretofore unpublished information made available by the two U.S. manufacturers of hex.

Hex was used in the past in large quantities for the production of numerous important organochlorine pesticides, including chlordane, aldrin, dieldrin, heptachlor, and endrin, as well as such minor but well-known pesticides as mirex and Kepone. At present, only Pentac^{R} and endosulfan are produced in significant quantities. The other current major use of hex is in the manufacture of flame retardants for plastics and polymers, a large and still growing market.

Since hex is basically a chemical intermediate with essentially no end uses of its own, hex concentrations in the environment should be negligible, and limited data suggest that this indeed is the case. Probably contributing to this is its ready disappearance through hydrolysis and photolysis. Due to its infrequency in the environment and its low profile as an intermediate, there have been few studies of the behavior of hex in the environment or in biological systems. Data on chronic exposures are especially lacking.

Very little is known regarding potential hex exposures to the general public through ingestion of contaminated food or water. Hex has been detected in waters near points of industrial discharge and in a few samples of indigenous fish, but elsewhere there appears to be almost no information on concentrations in surface waters, or in foods. The heaviest and most chronic exposures to hex undoubtedly occur among persons engaged directly in the manufacture of hex and among production workers fabricating hex-containing products. Inhalation is the primary mode of occupational exposure.

Extremely limited data are available concerning the effects of hex exposure on humans. A recent incident in which sewage treatment plant workers were exposed accidentally provides some information on acute responses, however, no systematic epidemologic studies of chronically exposed individuals have been reported.

Animal studies have demonstrated that hex is quite toxic via oral, dermal, and inhalation exposures. Chronic inhalation experiemnts have shown that inhalation of less than 1 ppm hex produced fatalities as well as a variety of pathological changes in several species of animals. To

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date, satisfactory subchronic and chronic oral toxicity studies have not been completed. Several attempted studies have failed to establish an oral dose which could be tolerated without mortality over an extended period of time. Similarly, little is known about the metabolism of hex. It appears that the compound is readily absorbed by the lungs, skin and stomach tissues and fairly rapid excretion occurs through the urine, feces and possibly through the respiratory tract. Standard toxicological tests for mutagenicity and teratogenicity have reported negative results, however once again, the extreme toxicity of the compound restricted the dosages used in these tests to extremely low concentrations of hex. These tests suggest that outright toxicity, rather than chronic effects, is perhaps the critical effect of hex, even at very low doses. Evaluation of the potential carcinogenicity of hex has not been possible due to the absence of chronic animal studies and epidemiologic studies.

This report was submitted in fulfillment of Contract NO. 68-03-2608 by Battelle's Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from September 21, 1977 to September 30, 1978, and work was completed as of October 31, 1978.

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We also wish to express our appreciation for the cooperation annd support received from the EPA staff on the Health Effects Research Laboratory during the preparation of this document, particularly to Dr. Jerry F. Stara, the project officer, who provided needed support and encouragement throughout the program. He was ably assisted by Bonita M. Smith and Karen L. Blackburn. The support of Dr. John R. Garner, Director of HERL, was much appreciated.

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1.0 GENERAL SUMMARY/ENVIRONMENTAL ASSESSMENT

Hexachlorocyclopentadiene, hereafter referred to as hex, is a highly reactive, highly chlorinated compound, which is the key intermediate in the manufacture of many important organochlorine pesticides and flame retardants for organic polymers. As such its production has been significant, touching 22.5 million kg (50 million pounds) per year at times.

Hex is an important chemical intermediate, but it has essentially no end uses of its own. Thus, hex concentrations in the environment should be negligible, and in general this is indeed the case. Small concentrations of hex were occasionally present as impurities in pesticides made from it, and some entered the environment this way. However, the most likely route for its entry into the environment arises from the manufacturing process, of either hex or products made from hex. These in fact represent the only documented sources of hex in the environment.

Because of its infrequency in the environment, and the low profile it maintained as a chemical intermediate, there have been but few studies of its behavior and data are exceedingly limited; most of the data contained in this environmental review represent laboratory investigations. However, by the same token, hex has heretofore not presented a major environmental problem, except in isolated instances such as the disposal of hex manufacturing wastes to the Louisville, Kentucky, sewer system.

This introductory chapter will provide a general summary of the more detailed coverage provided in the subsequent chapters. As indicated above, the sparseness of the available data precludes a very detailed treatment. Most of the experimental studies which have been conducted on hex have been performed by the Hooker Chemicals and Plastics Corporation and the Velsicol Chemical Corporation, the only two U.S. producers of hex. Without the data which these two firms have made available, this review of the environmental effects of hex would have been much more limited.

In the interest of organization, the topics will be reviewed and commented on in the order in which they appear in the document.

1.1 TECHNOLOGY OF HEXACHLOROCYCLOPENTADIENE

Hex, C_5Cl_6, is a pale yellow nonflammable liquid having a very pungent odor, soluble in a number of organic solvents but with a very low solubility, ca. 2 ppm, in water. Its boiling point is 239 C (462F) and it is sufficiently volatile at ambient temperatures to have a tendency to disperse to the atmosphere. This tendency to volatilize may also explain why its presence is found in so few environmental samples.

Its two double bonds make it a highly reactive compound which readily undergoes substitution and addition reactions. Its versatility is based upon its reactivity as a diene with a variety of olefins and polynuclear aromatic hydrocarbons in the Diels-Alder reaction. It is a key ingredient in the production of the cyclodiene group of chlorinated pesticides, including a number which had a large commercial market, e.g. chlordane^{R}, aldrin, dieldrin, heptachlor, isodrin, endrin, mirex, and Kepone^{R}. Environmental considerations have led to the banning, suspension, or severe restriction of the use of these pesticides. Only two hex-based pesticides have escaped these restrictions, and are still freely used, endosulfan (Thiodan^{R}), a broad spectrum insecticide, and Pentac^{R}, an acaricide used primarily for the control of mites in greenhouse cultivation.

Hex, unlike some of the pesticides derived from it, degrades rapidly by photolysis, giving water soluble degradation products. Tests on its stability towards hydrolysis at ambient temperature indicated a half-life of about 11 days at pH 3-6, which was reduced to 6 days at pH 9. In an aqueous solution, hex can disappear after as little as 30 minutes of photolysis.

Producers specifications for commercial technical hex call for 97.5 percent minimum hex content; principal contaminating chlorine compounds are hexachlorobutadiene (C_4Cl_6), and octachlorocyclopentene (C_5Cl_8). Of the impurities present in commercial hex, hexachlorobutadiene is the most important, since it goes through a Diels-Alder reaction unchanged and may carry through to the product; its toxicity is indicated to be greater than that of hex.

Commercial hex is purified by distillation, with higher-boiling compounds such as hexachlorobenzene (C_6Cl_6) and octachlorocyclopentene reporting in the distillation residue. Disposition of these by-products can create some environmental problems unless properly handled; octachlorocyclopentene appears to be less toxic than hex on the basis of some rather limited data.

Hex has only two major types of uses. Its use in the manufacture of organochlorine pesticides has already been mentioned. Because of the restrictions which have been placed upon the major members of this family, this use has decreased significantly from that of earlier years. How much is difficult to state quantitatively, since production or sales data are unavailable, but the main insecticides mentioned were each estimated to consume 2,000 to 5,000 metric tons/year 10 to 15 years ago, and chlordane was two or three times this. Thus, current use for pesticides must be only a fraction of this.

The principal current use of hex is in the manufacture of flame retardant compounds for incorporation into plastics and polymers to confer flame retardant properties on them. The major derivative used for flame retardants appears to be chlorendic anhydride and chlorendic acid, made by reacting equimolar quantities of hex and maleic anhydride. Hex can be reacted with numerous dienes to form other flame retardants, generally containing from 55 to 70 percent chlorine.

Beyond these two main uses of hex are only very minor uses, consuming but insignificant quantities of hex.

Estimates of current annual production rates for hex are uncertain and variable, as low as 7,200 tons to as high as 25,000 tons per year. The higher figure may be suspect since one of the two producers is not currently manufacturing hex.

1.2 HEXACHLOROCYCLOPENTADIENE AND THE ENVIRONMENT

Very little is known about the behavior of hex in natural aquatic and terrestrial ecosystems. In particular, information on the fate and transport of this compound is very sketchy. To date only one study has addressed these issues. Laboratory studies of microorganisms, insects, and fish have quantified several aspects of acute toxicity of hex using a rather wide range of test organisms. Unfortunately, there are insufficient data at present for assessment of the potential for chronic effects and its persistence in the environment.

Hex is extremely effective as a bacteriocide; 10 ppm hex was twice as effective in killing the common sewage bacteria *Salmonella typhosa* and fecal coliform than equivalent concentrations of chlorine. At the same concentration, hex appears to exhibit little toxicity to soil microbes, however. Tests for mutagenic activity (e.g., the Ames Mutagenic Assay) using several strains of *Salmonella typhimurium* indicated that although hex had a repressive (toxic) effect on the test organisms, it was not judged to be mutagenic.

Static bioassays showed a high level of acute toxicity to *Daphnia* in that 1 ppm hex was lethal to 50 percent of the organisms. Similarly, an LC_{50} of only 2.3 ppm was reported for mosquito larvae.

Based on current data, it is difficult to draw any general conclusions concerning the dosage required to produce acute toxicity to fish. An examination of the results of various bioassays shows large discrepancies in LC_{50} values reported for aquatic species. For example, trout and bluegill appeared to be able to tolerate much higher concentrations of hex than fathead minnows or fathead minnow larvae. Median tolerance for the trout and bluegill were reported to be 20-35 ppm, whereas the LC_{50} for fathead minnow larvae was less than 10 ppb. Such differences in sensitivity may relate in part to interspecies differences, life stage of the test organism, characteristics of the test (e.g., static or flow through systems) and water conditions such as hardness and temperature, or more likely some combination of these factors. Further replication of these tests will be necessary before recommendations can be made concerning safe concentrations of hex in natural waters. Rather preliminary evidence suggests that hex may accumulate in fish tissues, but this has yet to be demonstrated. Model ecosystem studies indicate the potential for biomagnification through the food chain, however, the probability of occurrence in the natural environment cannot be adequately evaluated, since essential information on the quantities of hex reaching aquatic ecosystems and the behavior of the compound under natural conditions is lacking.

Even less is known about the effects of hex in the terrestrial environment. It appears that soil conditions such as moisture and organic content play a decisive role in determining toxicity to insects. Studies utilizing radiolabelled hex applied to soil showed substantial losses of recoverable ^{14}C-hex; much of this loss was presumably due to volatilization.

1.3 ANIMAL TOXICITY

The classic studies of hex toxicity to mammals were conducted in the mid-1950's by Treon, et al. This series of investigations reported on both acute and subacute toxicity of hex to various species of mammals under a variety of exposure regimens. Oral, dermal, and inhalation modes of exposure were included in Treon's experiments. More recent proprietary studies of the oral and dermal toxicity have become available. In general, these findings agree remarkably well with those of Treon. Toxicologic mammal studies of hex subsequent to the 1950's could not be located in the open literature, probably due to the rather low profile of the compound relative to other pesticide chemicals.

Oral LD$_{50}$ values for hex lie in the range of 420-620 mg/kg in rabbits and about 500 mg/kg for rats. Thus, in terms of acute toxicity to rats, hex is intermediate between Kepone (LD$_{50}$ 95-140 mg/kg) and mirex (LD$_{50}$ 365-740 mg/kg), two closely related pesticides. In contrast to Kepone and mirex, hex is nearly as toxic via dermal application as it is following oral administration.

Hex also is an extremely potent irritant and accordingly has been classified as an "extreme irritant and corrosive substance" based on eye irritation tests as specified by FDA under the standards set by the Federal Hazardous Substances Act.

Inhalation tests indicate that hex vapors were extremely toxic to all four species of animals (guinea pigs, rats, mice, rabbits) employed in Treon, et al's tests. In fact, these tests reported that hex was more toxic than either phosgene or carbon tetrachloride. Despite some interspecies differences in sensitivity, inhalation of relatively low levels of hex (1.5-3.2 ppm) was fatal to half of the test animals following a seven hour exposure. Subchronic tests exposed mice, rats, guinea pigs, and rabbits to 0.34 ppm in air for 7 hours per day, 5 days per week. None of the rats or mice survived more than 20 such exposure periods. High mortality rates were observed at this dosage among rabbits as well. Yet another portion of Treon, et al's studies attempted to examine chronic oral toxicity. Rats and rabbits given various dosages ranging between 180-2000 mg/kg were fatal within such a short period that the investigators were unable to establish an oral dosage which could be tolerated without mortality over an extended period. A Soviet study reported that oral administration as little as 20 mg/kg for 6 months was fatal to 20 percent of white rats.

Pathologic examination of the hex-treated animals revealed similar types of abnormalities following oral, dermal and inhalation modes of exposure. Diffuse degenerative changes in the brain, heart, liver, kidney and adrenal glands were characteristic; the extent of damage varied directly with dosage and duration of exposure. Prolonged intermittent exposure to as litle as 0.15 ppm of hex vapor induced slight degenerative changes in the liver and kidneys of all species of animals tested.

Only two studies addressing the pharmokinetics of hex could be located. One of the studies exposed rats to various doses by gavage; a second portion of the same study examined guinea pigs exposed to varying doses of hex via dermal application. Hex was readily absorbed through the stomach tissues and through the skin. At necropsy both species of animals showed pathologic and histopathologic findings suggestive of excretion through the lungs. The second study examined absorption, metabolism, and excretion of hex following a single dose of radiolabelled hex. Assay of urine and feces of rats confirmed the presence of the labelled compound in both media. Furthermore, at least four metabolites were discovered in the urine although the exact identity of these metabolites is unknown at the time of this writing.

The same study revealed that urine represents an important route of excretion of hex. Seven days after administration, approximately one-third of the total administered dose had been excreted in the rats urine. Likewise, fecal excretion accounted for as much as 10 percent of the original dose. The body organs themselves retained only minute traces of the ^{14}C-hex, suggesting that less than 1/2 of the total dose could be accounted for but must have been eliminated by routes other than the urine or feces. Thus, this study also provides indirect evidence that excretion through the respiratory tract may be the primary mode of elimination of hex. Obviously, such a suggestion must be regarded as tentative pending confirmation from further studies.

Tests for teratogenicity (a pilot teratology study in rats) and mutagenicity (a dominant lethal study and several short term *in vitro* tests) have been reported as negative, however, it should be noted that extremely low doses were employed due to the toxicity of hex to the test organisms. Thus, the predictive validity of the results at higher dosages is uncertain.

Presently available data do not permit evaluation of the carcinogenic potential of hex. The required chronic animal studies have not been conducted, nor have any epidemiologic studies reported on cancer or on any other chronic effects in humans.

Due to its suspect chemical structure (based on the structural-activity relationships mentioned above), its widespread use an an intermediate in many cyclodiene pesticides and the relative lack of data on the effects of chronic exposure, hex has been selected for testing as part of the National Cancer Institute's test program. Hoepfully, further testing will clarify both the carcinogenicity issue and provide needed data on the chronic effects of hex on one or more mammalian species.

1.4 HUMAN TOXICITY

Extremely limited data are available concerning the effects of hex exposure on humans; no systematic epidemiologic studies of individuals chronically exposed to this compound have been reported to date. Consequently, most of what can be said about human health effects is based on inferences from animal studies and a few isolated incidents of accidental human exposure. Potential modes of human exposure are uncertain at this time. In particular, it is unknown whether oral exposure (e.g., through ingestion of hex-contaminated drinking water) constitutes a significant source of human exposure. Dermal and inhalation exposures undoubtedly occur among workers directly engaged in hex manufacture and probably among those engaged in the formulation of other related pesticides where hex may be present as an impurity. Recently, a group of sewage treatment plant workers were exposed to acutely toxic levels of hex arising from the clandestine disposal of large quantities of the compound to the Louisville, Kentucky, municipal sewer system.

Based on the findings of animal studies in which prolonged intermittent exposure of animals to hex concentrations as low as 0.15 ppm induced slight degenerative changes, prudence would dictate strict limitation of human exposure. Persons having opportunities for skin and respiratory contact with hex should be equipped with, and trained in the use of appropriate protective clothing and respiratory protection. The present Threshold Limit Value (TLV) for industrial exposure is set at 0.01 ppm or about 7 percent of the lowest vapor concentration shown to produce chronic toxic effects in laboratory animals.

Most people are capable of detecting the presence of hex in air at concentrations as low as 0.33 ppm by its pungent odor. Experimental studies have shown that some individuals could detect as little as 0.15 ppm. Laboratory workers developed headaches following incidental exposure to relatively low concentrations of hex vapor present in respiratory chambers used in animal experiments even after evacuation of the hex contaminated air and flushing with clean air. Although air samples were not taken during these episodes, a reasonable estimate would place the concentration which elicited the headaches in the 0.15 to 1.0 ppm range.

A single documented incident of acute human exposure occurred at the Morris Forman Wastewater Treatment Plant in Louisville, Kentucky. The problem apparently began in March, 1977, when an unknown odoriferous sticky material began entering the plant and gummed up equipment in the Screen and Grit Building of the plant. Workers attempting to remove the sticky material by steam cleaning experienced severe irritation of the eyes, nose, throat, lungs, and skin. Several of the men required medical treatment for these symptoms, but none were hospitalized.

2.0 TECHNOLOGY OF HEXACHLOROCYCLOPENTADIENE

2.1 CHARACTERIZATION OF HEX

2.1.1 Physical Properties

Hex is a pale yellow nonflammable liquid having a very pungent odor. It is soluble in all proportions in acetone, carbon tetrachloride, ethanol, and hexane at 25 C. (Forette, 1977). The solubility of hex in water is 2.10 ppm at 25 C and 2.25 ppm at 35 C (Dal Monte and Yu, 1977). The vapor pressure, as determined by Lanksmen (1978), can be represented by the equation:

$$\text{Log VP} = \frac{(-3050.47)}{T\,^{\circ}K} + 9.03638$$

and is 0.063 mm at 25 C.

Typical data and product specifications drawn from Hooker (1964) and Velsicol (1975) product bulletins are:

Grade:		Synthesis
Specifications:	Assay Hex, Minimum	97.5 percent
	Chlorine Content	78 percent
	Tetrachlorocyclopentadiene	0.6-0.8 wt. percent
	Octachlorocyclopentene	1.7-1.9 wt. percent
	Specific Gravity, 15.5/15.5 C	1.700-1.715
	Free Chlorine, ppm maximum	2.5
	HCl, ppm maximum	20
	Iron, ppm maximum	3
Other Properties:	Molecular Weight	273
	Boiling Point, 760 mm	239 C
	Boiling Point, 10 mm	108 C
	Melting Point	9.5-9.9 C
	Freezing Point Depression	16.1 C/mol
	Viscosity, cps 25 C	7.8
	Flash Point (Open Cup)	None
	Lb/gal at 15.5 C	14.2
	Refractive Index n_D^{25}	1.5625 ±.001
	Surface Tension	47 dynes/cm
	Latent Heat of Fusion	2712 cal/gm mol

Fairly extensive investigations of the spectra of hex, its derivatives, and bicyclic structures containing the same moiety have been reported. (Idol, et al., 1955; McBee, et al., 1955; Ungnade and McBee, 1958; and Brooks, 1974).

Hex has an absorption band in the ultraviolet at 322 m and 323 m (log e = 3.17) in ethanol. Allylic chlorines replaced by hydrogens shift the maximum absorption to lower wavelengths but increase the molar absorptivity. The infrared spectrum of the diene has two bands in the double-bond stretching region at 6.24 and 6.3 nm (1603 and 1572 cm^{-1}), 6.225 and 6.35 nm (1606 and 1575 cm^{-1}), and three bands in the C-C stretching region at 12.45, 14.21, and 14.75 nm (803, 704, and 678 cm^{-1}). The Raman spectrum gives two bands in the double-bond region at 1606 and 1572 cm^{-1} (Ungnade and McBee, 1958).

2.1.2 Chemical Properties

The two double bonds make hex a highly reactive compound which undergoes substitution and addition reactions to provide a variety of intermediates, for example, acids, acid halides and anhydrides, esters, amides, ketones, diketones, quinones, nitriles, and other halogenated hydrocarbons. The versatility of hex is based upon its reactivity as a diene with a variety of olefins and polynuclear aromatic hydrocarbons (Whetstone, 1964, and Roberts 1958) in the Diels-Alder reaction. These products are generally 1:1 adducts containing a hexachloro(2.2.1)bicycloheptene structure. If the dieneophile contains multiple unsaturation, hex may form both mono-adducts and di-or poly-adducts. Most of the insecticides are mono-adducts. The flame retardant products are both mono and diadducts (Rosenberg, 1978)

Diels-Alder additions with hex are generally batch reactions in stirred glass-lined kettles at 50-150 C for 5 to 20 hours. Olefin is usually in excess and the reaction normally occurs at atmospheric pressure unless the olefin is low boiling. The products are usually isolated as bottom products after vacuum stripping or are crystallized.

The reaction of hex with maleic anhydride leads to chlorendic anhydride and by hydrolysis to chlorendic acid, a key intermediate in the manufacture of flame-resistant resins.

Although steric isomers are possible in the condensation of hex with olefin, the products invariably contain the substituent derived from the olefin in the endo position. Products with the substituents in the exo configuration, are formed in small amount, if at all (Whetstone, 1964). With polycyclic olefins, products of a more complicated nature are formed, for example, exo-endo, exo-exo-, endo-endo, and endo-exo stereoisomers. Hex adds reversibly to polynuclear aromatics such as naphthalene and anthracene to yield potential intermediates in the manufacture of dyes (Look, 1974). Reduction of hex by catalytic hydrogenation in the presence of platinum or palladium catalysts proceeds stepwise to give pentachlorocyclopentadiene, tetrachlorocyclopentadiene and finally cyclopentane (McBee and Smith, 1955). Dechlorination of hex with zinc dust and ethanol yields cyclopentadiene. Both hexachlorocyclopentadiene and its reduction products are decomposed by alkaline substances (Brooks, 1974).

2.1.3 Stability of Hexachlorocyclopentadiene

A number of studies on the stability of hex under various conditions have been conducted in the laboratories of the Velsicol Chemical Corporation.

The photolysis of an aqueous solution of ^{14}C hex with light from a mercury-vapor lamp (medium pressure mercury vapor) was investigated by Yu and Atallah (1977a). Of the total energy radiated from the lamp about 40 to 48 percent was in the ultraviolet portion of the spectrum, 40 to 43 percent in the visible, and the remainder in the infrared. Gas liquid chromatography of the petroleum ether extract from the photolysis showed that the hex degraded rapidly to water soluble products. No hex was detected in the aqueous solution following 30 minutes of photolysis.

Studies on the hydrolysis of hex at various pH's and temperatures were also conducted by Yu and Atallah (1977b). ^{14}C hex was used to study the stability of hex at pH 3,6,9, and 12 at 25 C. At pH 12 the half-life of hex was less than 2 hours. At 25 C, the half-lives were 9.2, 10.6 and 4.4 days, at pH 3,6, and 9, respectively. Thin layer chromatography (TLC) was used to separate the hex and its degradation products. Gas liquid chromatography was employed to confirm the ^{14}C hex spots from thin layer chromatography.

As noted above, the boiling point of hex is 239 C (462 F), and the vapor pressure at 25 C is only 0.063 mm Hg. On this basis hex would be characterized as a relatively non-volatile substance, and would not be expected to be particularly labile. However, field evidence indicates that this is not the case, and that it disappears rapidly from aqueous and terrestrial substrates. This is evidently true even in the laboratory. Whitacre (1978) reports that even hex standards in organic solvents will degrade under laboratory conditions. It is common practice in Velsicol laboratories to cover volumetric flasks containing hex solutions with aluminum foil and keep them refrigerated while not in use. Otherwise, losses of 50 percent of hex can occur within a few days.

2.1.4 Analysis

2.1.4.1 Commercial Hexachlorocyclopentadiene --

As noted earlier, commercial technical hex may contain a number of other chlorinated hydrocarbons. Wysocki and Rozek (1977) assayed a "typical" production lot sample of technical hex for tetrachloroethylene (TCE), hex (C-56), hexachlorobutadiene (C-46), hex ketone, octachlorocyclopentene (C-58), hexachlorobenzene (HCB), pentachlorobenzene (C_6Cl_5H) and mirex. Analysis was done by gas chromatography and component identifications confirmed by mass spectrometry. Summarized results are shown in Table 2.1. Hex manufactured by other procedures may contain slightly smaller or larger concentrations of contaminants.

2.1.4.2 Environmental Samples --

Analytical methods for hexachlorocyclopentadiene are similar to those used for other chlorocarbons. Gas liquid chromatography (GC) is the method of choice, generally using an electron-capture detector. The Pesticides Analytical Manual, Volume I; Methods Which Detect Multiple Residues (U.S. Department of Health, Education, and Welfare, 1977) gives detailed descriptions of methodology, instrumentation, interfering substances, and confirmatory tests for chlorinated pesticides, much of which is applicable to the analysis of hex. Retention time is the principal parameter used to discriminate between compounds with GC analysis. This is a non-specific characteristic, and accurate analyses with GC depend critically on careful techniques and rigid control of operating parameters.

Some of the potential difficulties in the analysis of samples containing hex have been identified by Eichler (1978):

(1) Previously developed analytical methods may not be applicable to the specific problem in question, e.g. new interferences may be present

(2) Hex adsorbs rapidly onto metal surfaces in a gas chromatograph. Thus, it is good practice to use only glass coated lines. It also absorbs to a lesser degree on glass surfaces so that the amount of glass wool plug in the end of the chromatographic column may affect the analysis

(3) The location from which the biological sample came must be considered since the analytical apparatus of choice may not be applicable

(4) In trace levels analysis, contaminants may cause an erroneous response which may be interpreted as the presence of the contaminant of interest

(5) Conscientiously avoided must be such well known analytical difficulties as glassware contamination, solvent impurity, and variations in solvent purity, sample storage, and instrumental difficulties such as reproducibility in injection technique

(6) Advances in quantitative analysis have pushed the detectability limits for organics down to the ppb or ppt level. This has complicated the analytical scheme because more impurities and contaminants are now detectable. At present the state-of-the-art for confirming the structure of this multiplicity of trace contaminants is gas chromatography/mass spectrometry.

TABLE 2.1 SUMMARY OF ANALYSIS OF TECHNICAL HEXACHLOROCYCLOPENTADIENE[a]

Component	Typical Retention Time (Min)	Average Percent[b]	
C_2Cl_4	1.2	0.09	
C_4Cl_6 (C-46)	6.3	1.11	
Hex (C-56)	8.5	98.25	
C_6Cl_5H	11.5	0.02	
C_5Cl_8 (C-58)	12.8	0.68	
C_6Cl_6	14.1	0.04	
Internal Standard	21.7	--	
Mirex	33.5	ND[c]	
		100.2	Total mass balance

[a] Source: Wysocki and Rozek (1977).

[b] On basis of GC areas, normalized to hex.

[c] Not detected

2.2 METHODS OF PREPARATION

2.2.1 Laboratory Preparation

Hexachlorocyclopentadiene, C_5Cl_6 (1,2,3,4,5,5-Hexachlorocyclopentadiene) has been prepared by several methods which are described in the review article by Ungnade and McBee (1958). These include:

(1) The chlorination of cyclopentadiene with 6 to 11 mol of aqueous 0.25-4.5 molar sodium or potassium hypochlorite at temperatures ranging from -5 C to +50 C. Solvents such as benzene, carbon tetrachloride, chloroform, hexane or isopropyl ether may be used in up to 10:1 ratio with the cyclopentadiene, but the reaction may equally well be conducted without solvent. Side reactions can be minimized by the addition of 1 mol percent (based on the cyclopentadiene) of sodium sulfamate (Kleinman, 1953) or an emulsifying agent such as sodium lauryl sulfate (Lidov, et al., 1952). After about 20 minutes of reaction, the organic layer is separated and fractionated *in vacuo* to give a 55 percent yield of hex, boiling point 60 to 62 C/1 mm. The main by-product of this synthesis is 1,2,3,4,5 pentachlorocyclopentadiene which on standing gives a dimer, melting point 214 C. Compounds such as 1,4,5,5-tetrachlorocyclopentadiene can also be isolated from the low boiling material preceding hex.

(2) The condensation of hexachloropropene and dichloroethylene with aluminum chloride (Prins, 1937; and Prins et al., 1946).

(3) Stepwise condensation of trichloroethylene with chloroform followed by dehydrochlorination with aluminum chloride. The isolated intermediates in the reaction are hexachloropropane and pentachloropropylene (Prins, 1946).

(4) The dechlorination of octachlorocyclopentene by heat and catalysts or with hydrogen and platinum. Maude and Rosenberg, (1956) obtained about 90 per yield of hex when vapors of octachlorocyclopentene were contacted with a catalytic surface selected from the group consisting of nickel, cobalt, nickel chloride, cobalt chloride, and mixtures thereof in a reaction maintained at about 400-550 C. McBee, et al., (1955) obtained a 49 percent yield of hex from the hydrogenation of a mixture of octachlorocyclopentene, alcohol and platinum catalyst.

2.2.2 Commercial Manufacture

Rosenberg (1978) in his appraisal of past and current commercially attractive methods for manufacturing hex, provides information on the status of raw materials and recoverable by-products and distillation residues. The following comments can be made, based on his assessment of the above three parameters.

The choice of the starting material depends on a combination of technical and economic factors. The first report on the preparation of hex was published by Straus, et al., (1930). In their process, cyclopentadiene is reacted with an alkaline hypochlorite solution to give a crude hexachlorocyclopentadiene which also contains some dimeric by-products. The hex can be recovered by fractional distillation under reduced pressure. This procedure was used by Velsicol in developing their initial group of hex-based insecticides and the crude material was reportedly used to make technical Chlordane^R.

Exhaustive liquid phase chlorination of a cyclic C-5 hydrocarbon will provide octachlorocyclopentene (C-58) as the chlorination end product. This compound can also be made in low yield by liquid phase chlorination of various aliphatic C-5 and C-6 starting materials.

Newcomer (1953) described the conditions for making hex from trichlorethylene (C_2HCl_3) and carbon tetrachloride (CCl_4). Krynitsky and Bost (1974) reported the thermal vapor-phase dechlorination of octachlorocyclopentene (C-58) at a temperature of 470-480 C to yield hex. A procedure employing the preparation of C-58 by liquid phase chlorination of a cyclic C-5 hydrocarbon followed by vapor-phase dechlorination of C-58 to hex was developed subsequently and used commercially by Shell Development Company and is apparently the basis of the process used in their plant at Pernis in the Netherlands.

Various C-5 hydrocarbons can be used to prepare hex by a variety of vapor-phase chlorination processes. Because of the large heat of chlorination, it is usual to prepare a partially chlorinated material by a liquid phase reaction to be used as a feed to the vapor-phase reactor. If the parent hydrocarbon is normal or isopentane, a catalyst is needed in the vapor-phase reaction to obtain a high yield. No hex is obtained from neopentane. This process was developed by Hooker and operated successfully for many years. The crude product is a mixture of fully chlorinated materials. The by-products are mainly chlorocarbons formed by chain-rupture, such as carbon tetrachloride (CCl_4), tetrachloroethylene (C_2Cl_4), and hexachlorobutadiene (C_4Cl_6). Hexachlorobenzene (C_6Cl_6) may also be present. In the absence of a catalyst, these perchlorinated by-products may amount to as much as 50 percent by weight of the crude product. With the proper catalyst, the amount may vary from 5-15 percent of the crude product.

With the increasing availability of dicyclopentadiene from the thermal cracking of petroleum fractions in the late sixties, the use of this alternate raw material became attractive. This material is readily cracked to form cyclopentadiene which can be chlorinated in the liquid phase to obtain tetrachlorocyclopentane or more highly chlorinated cyclopentanes. Subsequent thermal or catalytic vapor-phase chlorination at elevated temperatures produces hex. Under closely controlled conditions no significant by-products are formed (except for the co-produced C-58). Under severe conditions various side reactions are observed. It is postulated that transient C_2Cl_2 free radicals are produced, as evidenced by the by-products which are formed. These free radicals may either trimerize to form C_6Cl_6, dimerize and chlorinate to form C_4Cl_6, or chlorinate to form C_2Cl_4. The concentration of these by-products increases at high reaction temperatures.

In summary, in commercial operation crude hex may thus contain the following chlorocarbons in varying proportions: CCl_4, C_2Cl_4, C_4Cl_6, C_5Cl_6, C_5Cl_8, C_6Cl_6. No C-3 chlorocarbons are present, since the perchlorinated compound C_3Cl_8 fragments to form CCl_4 and C_2Cl_4. Trace compounds containing residual hydrogen may also be present, but the amount is usually negligible.

Fractional distillation of crude hex usually results in virtually complete separation of CCl_4, C_2Cl_4, and C_6Cl_6. The distilled product will contain varying amounts of C_4Cl_6 and C_5Cl_8 depending on the composition of the crude product and the efficiency of the distillation. Specifications for the commercial product permit small amounts of these by-products. For most commercial uses, no further treatment of the distilled product is needed. The CCl_4, C_2Cl_4, and C_4Cl_6 are recovered as an overhead fraction in the distillation system. If a cyclic hydrocarbon is used as the starting material, the amount is too small to have commercial value. The C_6Cl_6 together with some C_5Cl_8 is removed as a molten distillation residue. Pure C_6Cl_6 is a high-melting (229 C) material which has low solubility in the other chlorocarbons in the crude. The residue is usually solid or semi-solid at room temperature.

Of the impurities present in commercial hex, the most important is hexachlorobutadiene (C-46). Most hex derivatives are made by a Diels-Alder condensation reaction. Any C-46 present is unchanged in the process and may carry through to the product, although, since most Diels-Alder condensations are carried out in a solvent, the unreacted C-46 is removed with the solvent. Published data on the toxicity of hexachlorobutadiene indicate that it is more toxic than hex. Commercial products such as aldrin may contain as much as 3 percent C-46. The C-46 content will depend on the commercial source of hex. The commercial product made by Hooker since installation of the dicyclopentadiene process contains very little C-46.

McBee, et al., (1950 and 1953), and Maude and Rosenberg,(1953 and 1956) described a two-stage process for the preparation of hex from pentane, isopentane, or mixed pentanes. The hydrocarbon mixture is chlorinated photochemically in the liquid phase at 80 to 90 C until an average composition of $C_5H_5Cl_7$ with a density of 1.63 to 1.70 is obtained. The subsequently vaporized chloropentanes are passed in a vapor of chlorine over a Floridin catalyst maintained at 350 to 400 C followed by passage through an upacked section of tubing heated at 500 C. By controlling the thermal chlorination in this manner, octachloropentene is produced which at 500 C gives a better than 90 percent yield of hex.

2.3 USES OF HEXACHLOROCYCLOPENTADIENE

The major uses of hex have been in the manufacture of chlorinated organic pesticides, initially, and more recently in the manufacture of flame retardants for polymeric materials. Other miscellaneous uses have been relatively insignificant.

2.3.1 Pesticides

Hex has been the chemical intermediate used for the production of numerous chlorinated pesticides, several of which have enjoyed very large usage. The list includes chlordane, aldrin, dieldrin, heptachlor, isodrin, endrin, mirex, Kepone, endosulfan (Thiodan^{R}), and Pentac^{R}. The routes of synthesis of these and other pesticides from hex are shown schematically in Figure 2.1. With the exception of endosulfan and Pentac, both of which are in current use, the usage of hex-based pesticides has been banned, suspended, or severely restricted by governmental action.

Although yields in all reactions are good, they are not quantitative. There is reason to suspect that in some cases free hex may have been present in the marketed pesticide products. For example, technical dieldrin is an insecticidal product containing not less than 85 percent of dieldrin and not more than 15 percent of insecticidally-related active compounds; technical heptachlor consists of 72 percent actual heptachlor and 28 percent related compounds; chlordane contains about 60 percent of the isomeric alpha - and beta - chlordanes and 40 percent of insecticidally related compounds -- chlordene, heptachlor, and nonachloro analogs; technical endrin contains 95 percent of endrin (Whetstone, 1964).

2.3.1.1 Thiodan^{R}--

Endosulfan, (Thiodan^{R}) $C_9H_6Cl_6O_3S$; 6,7,8,9,10,10-Hexachloro-1,5,5a,9,9a-hexahydro-6,9-methane-2,4,3-benzodioxathiepin-3-oxide (I) is manufactured by the reaction of hex with 1,4-diacetoxy-2-butene, followed by hydrolysis of the diacetoxy derivative to the diol, and reaction of the diol with thionyl chloride (Frensch, 1957, as quoted by Brooks, 1974). The diol intermediate can also be made directly by the Diels-Alder condensation of butenediol with hex, the procedure used by Hooker (Rosenberg, 1978b).

Endosulfan is a broad spectrum insecticide useful for the control of pests of deciduous fruits, vegetables, and ornamentals, especially of aphids, leaf hoppers, and spittle bugs. (Hooker, 1975).

The technical material is a brownish solid, melting range 70-100 C, insoluble in water, stable toward dilute mineral acids and hydrolyzed rapidly by alkalies. It has an oral LD_{50} to the rat of 110 mg/kg. Technical endosulfan consists of about 4 parts of alpha-isomer, m.p. 108-190 C, and one part of beta-trans isomer, m.p., 206-208 C. The alpha-isomer, which is a somewhat more potent insecticide, is slowly converted to the more stable beta form at high temperatures. Both isomers

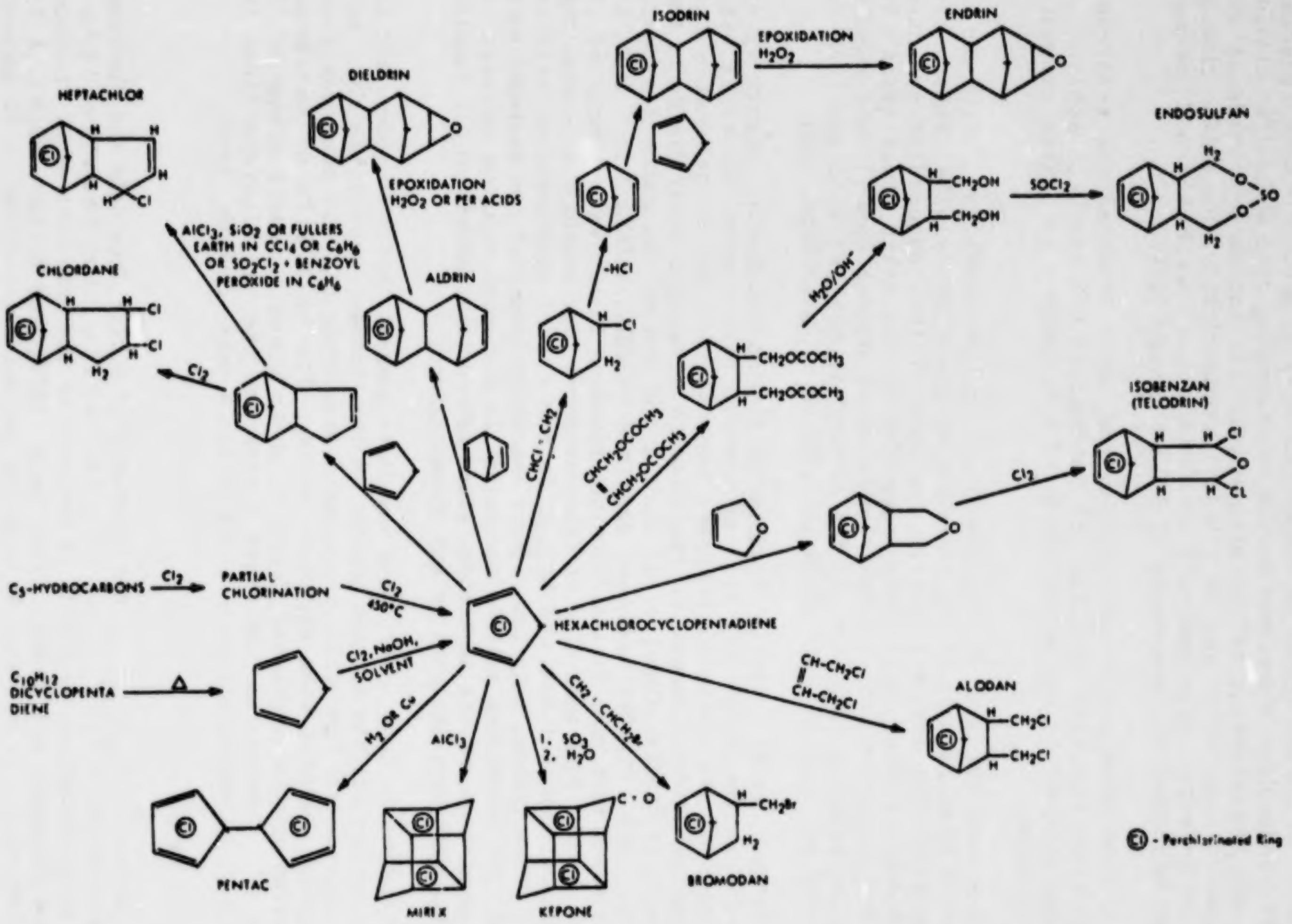

HEPTACHLOR
DIELDRIN
ISODRIN
EPOXIDATION H2O2
ENDRIN
ENDOSULFAN
CHLORDANE
AlCl3, SiO2 OR FULLERS EARTH IN CCl4 OR C6H6 OR SO2Cl2 + BENZOYL PEROXIDE IN C6H6
EPOXIDATION H2O2 OR PER ACIDS
ALDRIN
-HCl
H2O/OH-
SOCl2
Cl2
CHCl = CH2
CHCH2OCOCH3 = CHCH2OCOCH3
CH2OCOCH3
CH2OH
ISOBENZAN (TELODRIN)
C5-HYDROCARBONS
PARTIAL CHLORINATION
Cl2 450°C
HEXACHLOROCYCLOPENTADIENE
C10H12 DICYCLOPENTA DIENE
Cl2, NaOH, SOLVENT
CH-CH2Cl = CH-CH2Cl
ALODAN
H2 OR Cu
AlCl3
1. SO3 2. H2O
CH2 = CHCH2Br
C = O
PENTAC
MIREX
KEPONE
BROMODAN
Cl - Perchlorinated Ring

FIGURE 2.1. Synthesis of the diene group of chlorinated pesticides from hexachlorocyclopentadiene.

Source: Lawless, Von Rumker, and Ferguson (1972).

are slowly oxidized in air and in biological systems and rapidly by peroxides or permanganates to endosulfan sulfate, m.p. 181-182 C (Brooks, 1974). Endosulfan hydrolyzes to the corresponding diol and sulfur dioxide and when heated under reflux with methanolic sodium hydroxide, gives sodium sulfite which can be titrated iodometrically. The sulfur dioxide may be detected colorimetrically (red color) with Rosaniline and formaldehyde and the absorbance at 570 nm (Brooks 1974).

Another colorimetric analytical method, which involves the treatment of endosulfan with pyridine and methanolic KOH has been applied in residue analyses to the determination of both isomers in hexane extracts with cleanup.

The easy conversion of endosulfan into the parent diol, which can be detected by GC methods as the diacetate or bis-trimethylsityl ether, has been used for derivitization analysis of the compound in residues (Cochrane and Chau, 1971). Beta-endosulfan in the solid state exists in one modification with a highly symmetrical crystal form (I) and another with low symmetry (II). For (I) the SO band in the infrared spectrum is at 1,192 cm^{-1} and for (II) it is at 1,180 cm^{-1} (Maier-Bode, 1968).

Gorbach, et al., (1971) investigated the environmental stability of Thiodan in the water and soil of treated rice fields. Biotests with native fish and chemical analyses were carried out to determine the biological effectiveness of residues in submerged paddy fields in the vicinity of Pandaan (East Java) during the end of the rainy season in March, 1970. Thiodan residues declined rapidly, within 3 to 5 days, in the treated test rice fields. Terminal residues in the water amounted to 0.5-0.0 ppb. Fish were able to tolerate short time exposure to endosulfan concentrations 4 times the LC$_{100}$. In the mud of submerged as well as dried rice fields, only low residue concentrations (1.9 ppm maximum) were found. The increasing sulfate equivalent in the total residue pointed to decomposition of the pesticide. After appropriate extraction all samples in this work were analyzed by gas chromatography.

Studies in dogs, rats, mice and flies suggest that when endosulfan is ingested the sulfate appears briefly in the tissues, especially fat, and may appear in the milk of animals producing it, but such residues disappear rapidly when exposure ceases. The half-life of excreted products in the urine and feces of sheep given a single dosage of 14 mg/kg of ^{14}C-endosulfan was about 2 days and the radioisotope level in milk fell to negligible proportions within 4 days. (Brooks, 1974).

2.3.1.2 Pentac --

Pentac ^{R}, $C_{10}Cl_{10}$, is the trademark of Hooker Chemicals and Plastics Corporation (1968) for the acaracide bis-(pentachloro-2,4-cyclopentadien-1-y-1). It is prepared by the reductive dechlorination of hex (Ungnade and McBee, 1958; Ladd, 1956; and Rucker, 1960). A 73 percent yield is obtained by coupling two molecules of hex in 80 percent ethanol or methanol at ambient temperature with cuprous chloride or powdered copper bronze in light petroleum solvent (b.p. 100 C) or by refluxing with copper powder in toluene (Ungnade, and McBee, 1958). Reduction of hex with hydrogen at atmospheric pressure using a palladium on carbon catalyst gives about a 20 percent yield of Pentac (Brooks, 1974).

Pentac is a tan crystalline solid, m.p., 122-123 C, b.p. (decomposes at 250 C), vapor pressure 10^{-5} mm mercury at 25 C. It is insoluble in hot alcohol, aliphatic hydrocarbons, and moderately soluble in aromatic hydrocarbons. Pentac is stable towards aqueous acids and bases and can be safely stored for extended periods. (Hooker, 1968; Martin and Worthing, 1974). Hookers Pentac WP formulation contains 50 percent active ingredients. Pentac is recommended for mite control of greenhouse floral plants and nursery stocks, including roses, chrysanthemums,gardenias, carnations, azaleas, delphiniums, snapdragons, zinnias, and poinsettias. It is also effective on outdoor roses and nursery arbor vitae, including hemlock and spruce. Pentac apears to act by an interference with ovipositing of eggs by the female mites; initial results require 3 to 5 days. Application is at the rate of 8 ounces per 100 gallons of water which is sufficient to spray 2,000 to 3,000 mature bushes. Two applications should be made about 2 weeks apart. It has no insecticidal activity and is nonphytotoxic. (Allen, et al., 1964; Hooker, 1968).

The acute oral LD_{50} of Pentac (technical material and the emuslsifiable concentrate) for male albino rats is in excess of 3160 mg/kg; the dermal LD_{50} for albino rats also is greater than 3160 mg/kg. There was no evidence of systemic toxicity which could be attributed to percutaneous absorption. A single application of 3.0 mg of Pentac to rabbit eyes produces a slight irritation which subsided by the 6th day. (Hooker, 1968).

When heated at 130 C for several hours or when exposed to UV light or sunlight Pentac suffers pronounced loss of its acaricidal activity (Brooks, 1974). Pentac is analyzed by infrared spectroscopy with absorbance at 7.98 nm (Hooker, 1968; Martin and Worthing, 1974).

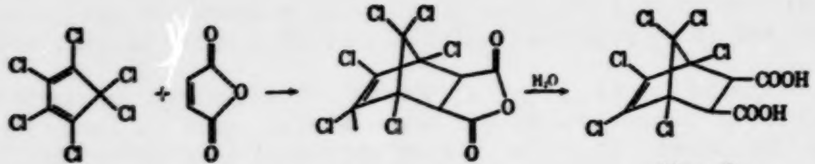
2.3.2 Flame Retardants

The increasing public emphasis on safety, accompanied by an expanding array of government regulations, has provided the impetus for a large and growing market for hex-derived chlorinated organic flame retardants. Flame retardant chemicals enable a material to resist burning when exposed to a relatively low-energy ignition source, such as a match, candle, or cigarette.

Hex-derived chlorinated organic compounds are used as flame retardants primarily in plastics, including polypropylene, polyethylene, nylon, thermosetting resin, rigid polyurethane foams, unsaturated polyesters, and other polymers, including epoxy resins (Sanders, 1978). Rough estimates of 1976 consumption of major alkyd coating resin reactants indicate that chlorendic anhydride finds limited use in fire-resistant paints for military applications (Chemical Economics Handbook, [illegible]977). Annual consumption probably does not exceed 250-300 tons.

These additives have the advantage of withstanding relatively high processing temperatures, but generally have to be used at high loading levels.

Examination of the chemical technology concerning flame retardants suggests that beginning about 1958 hex and some of its derivatives, notably chlorendic acid and some of its derivatives, began to become increasingly important in this field (Pattison and Hendersinn, 1971). Chlorendic anhydride ($C_9H_2O_3Cl_6$) can be prepared by heating equimolar amounts of hex and maleic anhydride in chlorobenzene at 140 to 150 C for 8 to 10 hours (Pattison and Hendersinn, 1971). Water is then added to the hot anhydride solution to effect hydrolysis and convert the anhydride to chlorendic acid monohydrate with better than 90 percent yield (after washing with chlorobenzene and water). Drying at 100 to 105 C yields an essentially anhydrous product of 99 percent purity:



chlorendic anhydride

chlorendic acid

Properties of the anhydride and acid are listed in Table 2.2. Table 2.3 lists the wide variety of polymer and coatings systems in which use of hex and chlorendic acid type flame retardants is recommended.

Hilado (1974) lists Hooker Chemicals and Plastics Corporation and Velsicol Chemical Corporation as manufacturers of a number of flame retardants, (Table 2.4), which are believed to be hex-based. Some clarification is needed concerning the present role of hex and $C_{10}Cl_{12}$ products manufactured by Hooker for fire-retardant applications. Although Dechlorane®* 510 and 4070 (mirex) are shown as products, these were discontinued on October 1, 1972 (Rosenberg, 1978b). Among products available from Hooker, the 1977 Buyer's Guide Issue of Chemical Week lists Dechlorane Plus 25, Dechlorane Plus 515 (chlorine content 65 percent) and Dechlorane 602 (chlorine content 69 percent). However, production of Dechlorane 602 and 604 has been discontinued (Rosenberg, 1978b).

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*Dechlorane is a trademark of Hooker Chemicals and Plastics Corporation

TABLE 2.2. DESCRIPTION AND PROPERTIES OF CHLORENDIC ANHYDRIDE AND CHLORENDIC ACID[a]

	Chlorendic Anhydride	Chlorendic Acid
Molecular weight	370.85	388.87
Percent chlorine	57.4	54.7
Appearance	White crystalline solid	White chrystalline solid
Melting point	240 - 241 C	Decomposes to chlorendic anhydride
Volatility	Very low at 25 C[b]	Very low at 25 C
Solubility (25 C) (g/100 g solvent)		
Benzene	40.4	1.1
Hexane	4.5	0.1
Acetone	127.0	144.0
Carbon tetrachloride	6.7	0.4
Linseed oil (raw)	19.3	9.4
Water	Hydrolyzes to chlorendic acid	0.4[c]

[a] Source: Adapted from Velsicol Commercial Development Technical Bulletin No. 524 (1961).

[b] Chlorendic anhydride sublimes at 90-100 C at a pressure of 0.5 mm mercury.

[c] Ca 7.0 at 94 C.

TABLE 2.3. POTENTIAL APPLICATIONS OF HEX AND CHLORENDIC ACID TYPE FLAME RETARDANTS[a]

Flame Retardant	Recommended for:																				
	ABS	Acrylics	Cellulose acetate	Cellulose acetate butyrate	Cellulose nitrate	Epoxies	Ethyl cellulose	Phenolics	Polycarbonates	Polyesters	Polyolefins	Polystyrene	Polyvinyl chloride	Urethane foam, rigid	Intumescent paints	Non-intumescent paints	Latex foam	Neoprene	Rubber	Textile coatings	Waxes
Dechlorane Plus 515 & 25	X					X		X		X	X	X	X			X	X		X	X	
Dechlorane 602[b]	X					X					X	X					X	X	X	X	
Dechlorane 603[c]									X		X										
Dibutyl chlorendate		X	X	X	X		X				X		X	X							X
Dimethyl chlorendate		X	X	X	X		X				X		X	X							X
Chlorendic acid						X				X				X	X	X					
Chlorendic anhydride						X				X				X	X	X					

[a] Source: Modern Plastics Encyclopedia (1976-1977). Reprinted by permission of Modern Plastics Magazine. (c) McGraw - Hill, Inc. (1977).

[b] Production has been discontinued.

[c] Did not achieve commercial status.

TABLE 2.4. HEX-BASED FLAME-RETARDANTS[a]

		Chlorine, Percent
	Hooker Chemical Corporation	
Dechlorane plus 25		65.1
Dechlorane plus 515		65.1
Dechlorane 510 (mirex)[b]		78.0
Dechlorane 4070 (mirex)[b]		78.0
Dechlorane 602[b]		69.4
Dechlorane 603[c]		66.7
Dechlorane 604[b]		
C-56	(hexachlorocyclopentadiene)	78.0
HET acid	(chlorendic acid)	54.7
HET anhydride	(chlorendic anhydride	57.4
	Velsicol Chemical Corporation	
Douse 499		42.7 (32.0)[d]
Dimethyl chlorendate		51.1
Dibutyl chlorendate		42.5
Hex-cod	(hexachlorocyclopentadiene-cyclooctadiene)	55.0

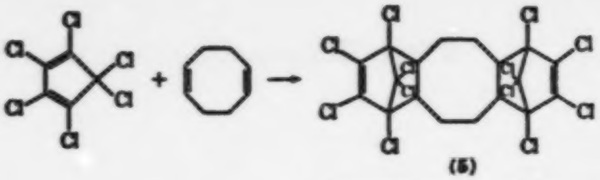
[a] Source: Adapted from Hilado. Reprinted with permission from Flammability Handbook for Plastics. (C) Technomic Publishing Company, Inc. (1974).

[b] Has been withdrawn from the market.

[c] Did not achieve commercial status.

[d] Also contains 32.0 percent bromine.

The halogen content of some of the current flame retardants that may represent products of more recent technology suggests that hex continues to be a key ingredient in their manufacture. For example, Pattison and Hendersinn (1971) report that bichlorendo (one of the earlier $C_{10}Cl_{12}$ products manufactured by Hooker) has been replaced in many applications by 1,5-bis (chlorendo) cyclooctane. This compound is prepared by a Diels-Alder reaction of 2 equivalents of hex and 1,5-cyclooctadiene as indicated in the equation below:



(5)

1,5-bis(chlorendo)cyclooctane

Bis (chlorendo) cyclooctane is used extensively with antimony oxide in flame retardant polypropylene and ABS (acrylonitrile-butadiene-styrene) formulations. Velsicol's Hex-Cod is probably obtained from the reaction of one equivalent of hex with 1,5-cyclooctadiene since the product contains 55 percent chlorine. Velsicol also manufactures Cytex B-56, a 1:1 adduct with 1,5 cyclooctadiene, followed by the additions of one mole of bromine to the remaining double bond.

Employing equivalents of diolefin and one or two equivalents of hex, the calculated chlorine content of the adducts resulting from each of several formulations is as follows:

Formulation	Product Chlorine, Percent
2 moles hex 1 mole cyclooctadiene	65.1
1 mole hex 1 mole cyclooctadiene	55.8
2 mole hex 1 mole butadiene	70.9
1 mole hex 1 mole butadiene	65.1
2 mole hex 1 mole cyclopentadiene	69.5
1 mole hex	

1 mole cyclopentadiene	62.8
2 mole hex 1 mole vinylcyclohexene	65.1
1 mole hex 1 mole vinylcyclohexene	55.8

Also, the reaction of 1 mole hex with 1 mole cyclopentadiene followed by the addition of 1 mole of bromine would be expected to give a product containing 32 percent bromine and 42.7 percent chlorine. These compositions correspond to the halogen contents of Velsicol's Douse 499. Thus, there seems little doubt that hex continues to be a key chemical in the preparation of flame retardants for plastics. In most cases, hydrocarbons have been modified with one or two equivalents of hex. Beyond this, chlorendic acid and the corresponding anhydride also are used in preparing flame-retardant resins and plastics.

Miller et al., (1976) investigated smoke and toxic gas emission from burning unsaturated polyesters (Table 2.5). Concerning chlorendic acid resin systems they observed that the smoke contained a high level of hydrogen halide (125 ppm per g of resin) but contained considerably less carbon monoxide than smoke from various halogenated resins.

2.3.3 Miscellaneous Uses

As discussed above, the current major uses of hex are found in the manufacture of pesticides, at present primarily endosulfan (Thiodan^{R}), and Pentac^{R}, and flame retardants for resins and plastics. There are many hex and chlorendic acid derivatives described in the patent and technical literature for which production and consumption figures are not available. Some of the recommended uses for these derivatives include bactericides, fungicides, plant growth regulators, weed eradicators, extreme pressure lubricants, rust inhibitors, flame-resistant composites with wood, rot-resistant additives in plywood, adhesives for rubber and plastics, and catalyst activators.

There is believed to only one registration ("Perma-Trim") for the use of hex as a contact herbicide; in this application it is recommended for use as a 0.5 percent solution along the edges of walks and driveways, where total eradication is desired. This use is quite minor, less than a ton per year.

In comparison with the markets for hex in pesticides and flame retardants, the consumption of hex at present for these minor uses is believed to be quite small, and in some cases can be only of informational and not technological interest. Examples of miscellaneous publications and patents concerning hex and hex-based derivatives are:

Enhancement of Flame-Resistance of Wool. Friedman, M., R. E. Whitfield and S. Tillin, 1973. Textile Research Journal 43; 212-217. Made wool

TABLE 2.5. SMOKE FROM VARIOUS HALOGENATED RESINS[a]

Resin System[b]	Smoke, D max/g.	HX,[c] ppm/g	CO, ppm/g
Atlac 711-054 (tetrabromobisphenol-A)[d]	177	24	144
Hetron 92 (chlorendic acid)[d]	93	>125	70
CoRezyn 925 (tetrachlorophthalic anhydride)[d]	124	>55	85
Dion 6125 (dibromohydrophthalic anhydride)[e]	94	15	168
Tetrabromophthalic anhydride (lab cook)[e]			
FR-1540	124	6	139
(dibromoneopentyl glycol) plus EG resin[e]	115	1.0	143

[a] Source: Miller, et al., Reprinted by permission of Modern Plastics Magazine. (C) McGraw-Hill, Inc. (1976).

[b] Atlac, ICI America, Inc.; CoRezyn, Interplastic Corp.; Dion, Diamond Shamrock Chemical Co.; FR-1540, Dow Chemical; Hetron, Hooker Chemical Corp.

[c] HX, X = Halogen

[d] Probably rated Class II by ASTM E84.

[e] Probably rated Class I by ASTM E84.

fabrics effectively flame-resistant by reaction with chlorendic anhydride in dimethylformamide.

Dyebath Application of Chlordenic Acid for Flame-Resistant Wool. 1974; Friedman, M., J. F. Ash, and W. Fong, Textile Research Journal 44; 555-556. Satisfactory flame resistance was obtained with chlorendic acid concentrations of about 8 percent.

Antiwear Formulation, Hammond, J. L., Conte, A. A., Jr., 1976. Wear 36(3); 387-90. Two weight percent dibutyl chlorendate included in a poly-(chlorophenyl)methyl siloxane fluid.

Hydraulic Fluid, Page, W. C. and Holbrook, G. W., Dow Corning Corporation Belgian Patent 839,860 1076. Six weight percent dibutyl chlorendate added to a liquid siloxane for usage in brakes and steering.

Solvent Extraction of Copper., Berger, S. A., 1976; Talanta 23(6), 475-479. The use of chlorendic acid was investigated as a function of pH in the extraction of cupric copper.

Dielectric Fluid, Brooks, W. T., 1976. Dow Corning Corporation, German Offen 2,608,447. To a liquid polyorganosiloxane was added 0.5 to 2.0 weight percent chlorendic acid or its esters containing C_1-C_{12} alkyl groups. Improved corona initiation voltage and corona extinction voltage were noted.

Accelerated Curing and Testing of Copolymer Finishes on Wood Panelings, Paszner, L., R. Szymani, and M. M. Micko, Holzforsch Holzverwert 27, 4-5. A mixture of tetraethylene glycol dimethylacrylate and chlorendic anhydride gave the highest hardness and chemical and abrasion resistance but had poor ultraviolet resistance.

Polymeric Photopolymerization Initiators, Wagner, H. M., J. S. Foster, Lowman, R. C., 1975. Research Disclosure 134, pp 19-21. Chlorendic anhydride was used in the preparation of a photopolymerizable ester.

Extreme Pressure Additives for Lubricating Oils, Fields, E. K., A. Steiz, Jr., Standard Oil Indiana. 1977. U. S. Patent 4,025,569.

2.4 PRODUCTION OF HEXACHLOROCYCLOPENTADIENE

Hex has been manufactured in the United States only by the Velsicol Chemical Corporation at Memphis, Tennessee, and by the Hooker Chemicals and Plastics Corporation, initially at Niagara Falls, New York, and in recent years at Montague, Michigan. In 1977 Hooker discontinued hex manufacture at Montague, making Velsicol's Memphis plant the only U.S. producer.

Although Hooker and Velsicol were the only U.S. producers of hex, the Shell Chemical Company was a major user of hex in the manufacture of organochlorine insecticides (aldrin, dieldrin, and endrin) at its Denver plant at Genk, Belgium, also produces chlorendic acid from hex (Rosenberg, 1978b). No information was identified on either imports or exports of hex or hex derivatives; neither is believed to have been a major factor in U.S. statistics.

Production statistics on hex are unavailable, and estimates are fairly broad, partly because production has risen and fallen with the usage of its major insecticide products. Pesticide production/sales estimates for 1962 and 1972 (Table 2.6) provide an indication of U.S. consumption of hex for these purposes.

Whetstone (1964) stated that the annual production of hex in 1962 for the preparation of cyclodiene insecticides must have been at least 22.5 million kg (50 million lb). Lu, et al., (1975) also concluded that the production in 1972 for the same purpose could not have been less than 50 million pounds. The above estimates of hex production for the preparation of cyclodiene insectides seem quite realistic for the period in question. Because of the restrictions which have been placed on the major chlorinated insecticides current production for these uses appears to be only a small fraction of these levels.

The other major use for hex was in the manufacture of flame-retardants. In 1968, approximately 7,725 metric tons (17 million pounds) of reactive flame retardants were used for unsaturated polyesters, of which about 5,000 metric tons (11 million pounds) were chlorendic acid. As shown in Table 2.7 the current consumption of flame retardants approximates 175,000 metric tons, of which chlorinated paraffins and chlorinated cycloaliphatics (the latter would include hex), chlorendic acid, and anhydride, and derivatives of both hex and chlorendic acid command a sizeable market. Chlorendic anhydride is the largest volume reactive chlorinated flame retardant used (Levek and Williams, 1976).

As reported in Chemical Economics Handbook (1976) approximately 3 million pounds of maleic anhydride were used in 1974 for the production of about 10 million pounds of chlorendic acid and anhydride. Since equimolar quantities of hex are used, and its molecular weight is about 2.77 times that of maleic anhydride, production of this quantity would require about 8.3 million pounds (4,150 tons) of hex.

The same source projects a growth rate of about 10 percent per year from 1974 through 1980 due to increasing use of flame retardant polyester resins for corrosion resistant ductwork and building panels, appliance parts, and insulation for electrical apparatus. This suggests that about 5 million pounds of maleic anhydride (and nearly 14 million pounds of hex) will be used in the manufacture of 16.6 million pounds of chlorendic acid and anhydride in 1980.

Aside from the general statement (above) on the use of chlorendic acid and anhydride in flame retardant polyester resin formulations, very

TABLE 2.6. PRODUCTION/SALES OF PESTICIDES MANUFACTURED FROM HEXACHLOROCYCLOPENTADIENE

Pesticide	Estimated Annual Total, Metric Tons (S.T.)			
	1962[a]		1972[b]	
Chlordane	2,250-4,500	(2,500-5,000)	11,340	(12,500)
Aldrin	--		4,500	(5,000)
Heptachlor	2,250		2,700	(3,000)
Endosulfan	450-900	(500-1,000)	910	(1,000)
Dieldrin	2,250-4,500	(2,500-5,000)	450	(500)
Endrin	2,250-4,500	(2,500-5,000)		
Mirex	--		--	
Pentac^R	--		45[c]	(50)[c]

[a] Whetstone, et al.,(1964).

[b] Lu, Po-Yung, et al.,(1975).

[c] Estimated current production.

TABLE 2.7 FLAME RETARDANTS USED IN PLASTICS[a]

Type	Thousands of Metric Tons, 1975	Thousands of Metric Tons, 1976
Additives		
Alumina hydrates	60.0	70.0
Antimony oxide	8.5	11.0
Boron compounds	4.0	4.7
Bromine compounds	9.0	9.5
Chlorinated paraffins and cycloaliphatics	30	35
Phosphate esters, nonhalogenated	18.7	21.0
Phosphate esters, halogenated	13.5	17.5
Others	5.0	6.0
Total	148.7	174.7

[a] Source: Adapted from Modern Plastics (1976). Reprinted by permission by Modern Plastics Magazine. (C) McGraw-Hill, Inc. (1977).

little information was found concerning the specific applications of these raw materials in coatings.

An estimated production figure of 7200 tons per year of hex has been reported for the 1975-1976 period (U.S. Environmental Protection Agency, 1977). This estimate may be low. Some industry estimates suggests 2.5 to 5 million kg/yr (5 to 10 million lb) production of both chlorendic acid and chlorendic anhydride, and total annual hex production of the order of 22 million kg (50 million lb).

Hex is sold as a distilled liquid of high purity in nonreturnable 55-gal lined drums (700 lb) and in 8,000 gal. tank cars. Hex is not extremely corrosive and can be stored and handled in steel without harming product or container, if moisture is rigorously excluded. However, to avoid possibility of iron contamination and corrosion, glass, nickel, or baked phenolic coatings are recommended (Hooker, 1964). It is classified as a Class B poison under DOT Regulations, and has a poisonous liquid NOS, Class B Freight Classification (Hooker, 1969).

3.0 HEXACHLOROCYCLOPENTADIENE AND THE ENVIRONMENT

In this section the effects of hex on microorganisms and on some aquatic and terrestrial biota are discussed. The amount of information is not large, and because there are very few field data on hex, the following evaluation is based almost entirely upon laboratory studies.

3.1 EFFECTS ON MICROORGANISMS

Hexachlorocyclopentadiene (hex) has been shown to have bactericidal properties and is reported to have germicidal activities towards many fungi (Cole, 1954). A static bioassay study of its effects on sewage effluent showed that hex is more toxic than chlorine in reducing bacterial counts (Cole, 1954). Ten ppm of hex reduced the total count from four million to less than 10,000 in two hours,a 90 percent reduction, whereas chlorine reduced the count by only 45 percent in the same interval. Both five and ten ppm hex were equally effective against coliform and Salmonella typhosa and resulted in a 90 percent reduction in one hour, while chlorine produced a 50 percent reduction. In contrast, Rieck (1977b) found no effects of hex at 15 ppm on bacterial populations, actinomycetes or fungi in a sandy loam soil. Rieck concluded that no significant deterimental effects on microbe populations would result from treating soil with high levels of hexachlorocyclopentadiene. The difference in results between Cole and Rieck may be due to the volatility, degradability, and adsorption of hex.

The Ames Mutagenic Assay conducted by Industrial Bio-test Laboratories, Inc., (1977) using four to five strains of Salmonella typhimurium indicated that hex is not mutagenic. The tests were made with and without metabolic activation, using concentrations of hex up to 2500 ug/10 ul added to the microbial assay plates. Concentrations greater than 10 ug/10 ul produced a possible cidal effect in all but one of the strains tested; a possible cidal effect occurred at 2500 ug/10 ul or greater in the remaining strain in the absence of metabolic activation. A repressive effect was noted in three strains at concentrations below 10 ug/10 ul in the absence of metabolic activation. Volitilate (volatile vapors) of hex was also tested on one strain using the vapor from hex concentrations of up to 2500 ug/10 ul and exposure times of up to two hours. Again no mutagenic effects were observed and the repressive effect was again noted.

3.2 EFFECTS ON AQUATIC BIOTA

3.2.1 Insects

Static bioassays using the waterflea, *Daphnia magna*, illustrate that hex can be lethal to daphnids (Union Carbide Environmental Services, 1977). Daphnids are found in nearly all types of freshwater habitats and are preyed upon by many species of fish; therefore they are an important food web organism. Acute 48-hour toxicity studies of *Daphnia* were made using filtered lake water. The concentration of the toxicant lethal to 50 percent of the test populations (LC_{50}) at 24 hours was determined to be 93.0 ppb (range 78.9-109.6; 95 percent confidence limits); the 48-hour LC_{50} was 52.2 (44.8-60.9) ppb. The no-effect concentration was less than 32.0 ppb. The LC_{50} and no-effect levels may be expected to vary with different temperatures, species, and water qualities. Lu, et al., (1975) reported an LC_{50} of 2.3 ppm for mosquito (*Culex pipiens*) larva.

3.2.2 Fish

Davis and Hardcastle (1959) conducted static bioassays of several hydrocarbon compounds including hex, for possible use as herbicides in Louisiana. The purpose was to evaluate the effects on common fish of representative responsiveness. The species selected for testing were bluegill (*Lepomis machorchirus*) and large mouth bass (*Micropherus salmodes*). A median tolerance limit (TLM) was established in soft water from Bayou de Siard, a quiet cut-off fed primarily by surface water. Both species showed obvious distress within 5 to 10 minutes after the introduction of hex; there was a loss of equilibrium and only slight response to touch; however, no deaths occurred in 24 hours in concentrations up to 500 ppm. At 48 hours, the TLM was 30 and 35 ppm for bluegill and large mouth bass, respectively; at 96 hours, it was 25 and 20 ppm.

In another reported bioassay, it was found that hex was lethal at 6 ppm to trout and bluegill in 15 to 30 minutes and lethal to sea lamprey in eleven hours. Effects at 1 ppm were roughly the same; at 0.1 ppm no effects were observed for up to 24 hours (Equitable Environmental Health, 1976). These lethal concentrations for bluegill are much lower than those reported by Davis and Hardcastle (1959).

In a study using early life stages of fathead minnows, (*Pimephales promelas*) in a flow-through bioassay system survival was significantly decreased at 7.3 ppb and above after 4 days of exposure. Most fish were killed in 4 days and all in 30 days in hex concentrations of 9.1 ppb. Growth of experimental and control fish was not significantly different at hex concentrations below 7.3 ppb. A toxicity curve shows that this compound is a non-cumulative poison (Spehar, et al., 1977).

The toxicity of hex on fathead minnows (*Pimephales promelas*) was tested by the U.S. Department of Health, Education, and Welfare in 1956 (cited in the report prepared for The Hooker Chemicals and Plastics

Corporation by Equitable Environmental Health, Inc., 1976). Recorded 24-, 48-, and 96- hour TLM values in hard water (emulsion) were 0.075, 0.059, and 0.059 ppm, respectively. These reported values are eight times higher than those found by Spehar, et al., for the same species (see above). Comparison of the results test results of Spehar et al. with those of earlier studies shows that all three of the compounds tested (hexachlorocyclopentadiene, hexachloronorbornadiene or heptachloronorbornene) were more toxic to fathead minnows in Spehar's (1977) study than to this same species and others exposed to these compounds for similar time periods. The authors attributed the lower values obtained in this test to the utilization of intermittent flow exposure systems and/or the use of more sensitive life stages of development for testing (Spehar et al., 1977).

In examining the findings of hex toxicity to fish, one finds discrepancies which at present cannot be explained. Differences in species tested, life stage of the test animal, the characteristics of the test (e. g. static vs. flow-through bioassays), and water conditions are factors which possibly contribute to the variation in results between studies. In addition, in many cases, the purity of the compound termed hex was not reported. Thus, differences in purity of toxicant might also account for variability in bioassay findings.

Hexachlorocyclopentadiene and other organochlorine compounds have been identified in aqueous discharges from a chemical plant in Michigan which manufactures hex. (Swanson, 1976). A 72-hour static bioassay of varying dilutions of the effluent from a plant outfall indicated that the LC_{50} was 61 percent effluent; a 100 percent effluent killed all fish in one hour and a 75 percent effluent killed all fish in 72 hours (DeKraker, 1976).

Yap, et al., (1975) studied the biochemical effects of various cyclodiene insecticides. The study involved the in vitro inhibition of fish brain ATPase activity by compounds such as aldrin, endrin, dieldrin, chlordane, heptachlor, and Kepone, (for all of which hex is a chemical intermediate). Hex itself, however, was not used in this study. Bluegills were used as the source of brain tissue. Of the 15 cyclodiene compounds tested, all except dieldrin, isodrin, endrin, pentachlorophenol, and mirex inhibited both Mg^{++} and Na^{+}-K^{+} ATPase. Dieldrin, isodrin, endrin, and pentachlorophenol stimulated Mg^{++} ATPase; mirex had little effect on either Mg^{++} or Na^{+}-K^{+} ATPase (Yap, et al., 1975). Although in vitro results cannot be translated into known toxicity values for organisms or extrapolated to hex this information suggests that hex may inhibit ATPase.

3.3 EFFECTS ON TERRESTRIAL BIOTA

Hexachlorocyclopentadiene has been used as contact herbicide on grasses and weeds (Cole, 1954) such as along sidewalks driveways, and fences. It reportedly has unusual fungicidal properties (Cole, 1954b) but Rieck (1977a) reported little effect on soil fungi (see above).

The specific effects of hex in the terrestrial environment remain largely unknown. Impacts of the compound can vary significantly with environmental conditions. Precipitation and wind can result in the transportation of the compound to unintended areas, with the possibility of high concentrations occurring. Concentrations of the substance can be reduced by volatilization; the rates of volatilization, in turn may be affected by temperature, moisture and humidity. Thus, concentrations and persistence can vary from time to time and place to place.

Soil conditions can have major influences of the toxicity of hex-related substances, (e.g. heptachlor, diazinon, dieldrin). Harris (1966,1972a, 1972b) has shown that these insecticides are more effective in moist soils than in dry soils and more effective in mineral soils than in soils with high organic content. A direct-contact toxicity test of technical chlordane and its components, one of which is hex, was conducted using crickets (*Gryllus pennsylvanicus*) and picture-winged flies (*Chaetopsis debilis*) (Harris, 1972b). Hexachlorocyclopentadiene proved to be the least toxic of eight compounds tested (technical chlordane, gamma-chlordane, alpha-chlordane, nonachlor, heptachlor, chlordene, 1-hydroxychlordene, and hex), showing no toxicity to either species at a one percent solution after 48 hours. Lu, et al., (1975) reported a LD_{50} of 565 ug/g for hex for house flies (*musca domestica*) following a topical application, a concentration much lower than that reported by Harris (1972b) for other insects.

3.4 TRANSPORT AND FATE

Hexachlorocyclopentadiene enters the environment primarily through discharges and emissions from pesticide production facilities; some also enters the environment through pesticides and compounds of which hex is present as an impurity, e.g. chlordane (Harris, 1972b). Once in the environment it may be transported by wind, surface and underground water, streams, and biota.

In December, 1975, hex was qualitatively indentified as a contaminant in the discharge of a pesticide production plant in Memphis. Later, (May, 1977) the compound was identified in the air near a Michigan pesticide production plant and in its aqueous discharge, and in fish tissue from the receiving stream (Spehar, et al., 1977). Hex has also been reported to be present in soil and bay sediments in the vicinity of a Virginia pesticide plant long after production was discontinued (Swanson, 1976). In addition, improper disposal of large quantities of hex resulted in widespread contamination of sewer lines and shutdown of the municipal sewage treatment plant in Louisville, Kentucky, as described in Section 5.2.

Evidence presented by Rieck (1977a) in an investigation conducted for Velsicol, indicated that vapors of ^{14}C-hex (and degradation products) envolved from treated soil (Maury silt loam), to the extent of 11, 13, 15, 16, 17, and 20 percent (cumulative) after passage of air over the samples for 1, 2, 3, 5, 7, and 14 days. One could, therefore, deduce that there is volatility from treated soil, and that the rate decreases with time.

An interesting aspect of this test was that a little more than half of the volatile products were trapped by hexane and a little less than half by a 1:1 ethanolamine solution, indicating that some polar metabolites were evolved (which were not extractable from the ethanolamine by hexane). Sohxlet extraction of the residual soils with hexane and 80 percent aqueous methanol extracted insignificant percentages of the applied ^{14}C. A total of 38 percent of the applied ^{14}C was unextractable as determined by combustion analysis.

In another experiment Rieck (1977c) applied ^{14}C-radiolabeled hex to soil under various conditions to determine the rate of degradation. At the start of the experiment, 93 percent of the applied substance was extractable. After 7 days less than 10 per cent of the applied ^{14}C-hex was extractable; 36 percent was extractable in autoclaved soil. Approximately 6.5 to 9 percent of the original ^{14}C-hex in the non-autoclaved soils was in the form of metabolized polar products after seven days and declined to about 3.5 to 5 percent of the total after 56 days. The polar products accounted for 3 to 4 percent of the total in autoclaved soil during the entire period. During the study the total ^{14}C-hex recoverable declined from 94 percent at the initiation of the study to 60-75 percent at seven days; 50-60 per cent was recoverable at 56 days. The loss of recoverable ^{14}C-hex was attributed to volatilization of the compound.

In this study soil which had been extracted was combusted, so that any residual but unextracted ^{14}C was measured directly as $^{14}CO_2$. Unextracted ^{14}C was found in these samples and thus was accounted for as a "bound" residue. Had it not been accounted for it would probably have been assumed to have volatilized.

Volatilization during extraction is also thought to be the reason why residue accumulations of hex have not been found in edible fish and why accumulation in fathead minnows was not demonstrated in the test by Spehar et al., (1977).

Lu,et al., (1975) investigated the environmental distribution of 'hex', chlordene, heptachlor, and heptachlor epoxide in food chain organisms in two laboratory model ecosystems and *in vitro* by sheep liver microsomes. In the first type of model ecosystem study ^{14}C-labeled 'hex' was added directly to the water at approximately 0.1 ppm and allowed to pass through a food web of plankton, daphnia (*Daphnia magna*), mosquito larva (*Culex pipien quinquefasciatus*), fish (*Gambusia affinis*), alga (*Oedogonium cardiacum*) and snail (*Physa sp.*). The transfer and degradation were observed over a 3-day period at 80 F (26.7 C). In the second type of model ecosystem study 5 mg of ^{14}C hex was topically applied from acetone solution to grass (*Sorghum vulgare*) growing on the terrestrial portion, simulating an agricultural application of 1.12 kg/ha (1.0 lb/ac). The model ecosystem was allowed to run for 33 days; maximum hex concentration in the water (0.031 ppm) was reached after 14 days. The plants were consumed by the salt marsh caterpillar larvae (*Estigmene acrea*) and the ^{14}C-labeled products entered the terrestrial portion as fecal products, leaf frass, etc. The organisms in the aquatic portion were the same as listed for the model aquatic system and the radiolabeled products were allowed to pass through the system over a 33-day period at 80 F with a 12-hr diurnal cycle and a 500 ft-candle illumination.

At the conclusion of both sets of experiments the radioactivity in water was extracted in ethyl ether and in the various organisms in acetone, and evaluated as total parts per million, and for relative amounts of degradation products by thin-layer chromatography, autoradiography, and liquid scintillation counting of the ^{14}C-labeled spots. The residual activity in the extracted substrates was determined by total combustion analyses as $^{14}CO_2$. The identification of the metabolites was confirmed by cochromatography and gas-liquid chromatography. Final aqueous hex concentration was 0.00024 ppm.

In the view of Lu, et al, hex showed considerable environmental stability, and from 22 to 50 percent of extractable ^{14}C detected in alage, snails, mosquito, and fish was present as hex. What were denoted as ecological magnification (EM) factors, based on the final hex concentration in the water were given as follows: alga, EM 340, snail, EM 929, mosquito, EM 1634 (The latter two appeared to be reversed in the text; the tabular data indicate mosquito to be EM 929 and snail EM 1634), and fish, EM 448.

As has been pointed out by Whitacre (1978), the authors appear to have departed from more generally accepted terminology in describing the increases in concentration as "ecological magnification". What appears to be happening here is bioconcentration, referring to the process whereby chemical substances enter aquatic organisms through gills or epithelial tissues directly from water. Bioaccumulation is a broader term referring to a process which includes bioconcentration but also includes any uptake from dietary sources. The term biomagnification is generally reserved to describe the process by which tissue concentrations of bioaccumulated chemical residues increase as these materials pass up the food chain through two or more trophic levels.

On the basis of the tissue concentrations presented by Lu, et al, biomagnifications are all less than five, and in one case less than one, i.e., a negative magnification. Additionally, although the substantial volatility of hex was recognized, the reported biocentration (ecological magnification) factors were based on the ratio of tissue concentrations to the residual aqueous hex concentrations after a 33 day exposure to 80 F and 500 ft-candles of illumination. As noted in Section 2.1.3, hex is subject to both photolysis and hydrolysis, so that final residual hex concentrations are quite probably not representative of the overall behavior of the system.

As suggested by Whitacre (1978), since the total ^{14}C in the system was originally all hex, residual extractable ^{14}C, which includes residual hex plus its degradation products, may better serve as an approximation of non-volatilized hex. Bioconcentration factors so calculated range between 25 and 80.

While natural conditions in the environment are much more complex than those in the model ecosystem, the study illustrates that hex does have the capacity for biomagnification in aquatic ecosystems. Since hex has no dispersive uses, the existence of this type of situation should be rare, however, it could occur perhaps as the result of a spill or a non-permissible discharge.

4.0 ANIMAL TOXICITY

4.1 ACUTE AND SUBACUTE TESTS

The classic studies on acute and subacute toxicity (31 weeks) were carried out in the 1950's by Treon, et al., (1955). Acute toxicity of hex by oral, dermal and inhalation routes of exposure was examined in a variety of animal species including guinea pigs, mice, rabbits, rats, and monkeys. In addition, the effects of subacute vapor inhalation (150 7-hour exposures over a seven month period) were also studied. Results of these tests are summarized below.

4.1.1 Oral Administration

Acute toxicity of hex was determined by Treon et al., (1955) by administering various dosages of a 5 percent solution of hex in peanut oil directly into the stomachs of several groups of rabbits and rats. The data on rabbits indicate that the lethal oral dose, administered as described above, lies in the range between 420 and 620 mg/kg of body weight.

Rats showed variation in minimum lethal dose depending on sex. Male rats were somewhat more sensitive in that the lethal dose was somewhat less than 280 mg/kg body weight whereas for females, the dosage causing death was greater than 280 mg/kg. The LD$_{50}$ for male rats was determined to be 505 mg/kg with 95 percent confidence limits of 387-623. It should be noted that very few of the test animals survived longer than a week after oral administration of hex.

The International Resesarch and Development Corporation (IRDC, 1968) conducted similar studies of the oral toxicity of hexachlorocyclopentadiene and octachlorocyclopentene to male albino rats. Each of the test compounds was mixed in corn oil and administered to the rats at dosage levels of 100, 215, 464, 1000, 2150 and 4640 mg/kg of body weight. Five rats were tested at each of the above dosage levels of each compound.

The dose which was lethal to 50 percent of the rats (LD$_{50}$) was determined to be 926 mg/kg for hex which is somewhat higher than that reported earlier by Treon, et al., (1955). The LD$_{50}$ value reported for octachloroyclopentene was 1470 mg/kg, indicating a somewhat lower toxicity for "octa" than for hex.

4.1.2 Cutaneous Administration

In this series of experiments, 93.3 percent hex was applied to the intact skin of rabbits using the technique of Draize, et al., described by Treon, et al., (1955). It was determined that the lethal dosage lies between 430 and 610 mg/kg body weight. Such a thing is remarkable in that hex appears to be just as toxic via dermal application as by ingestion.

The effects of sublethal concentrations were investigated in both rabbits and monkeys. In the case of the former, dosages as low as 250 mg/kg induced extreme irritation, purplish-black discoloration of the skin and subcutaneous edema. Although the skin lesions healed eventually, damage to the skin in the area of application persisted for many days and the damage varied in severity and extent with the amount (dosage) of the material applied.

A slightly different procedure was employed in the cutaneous exposures of the monkeys. In this case, a series of hex concentrations (0.001, 0.01, 0.1, 1.0 and 10.0 percent) dissolved in Ultrasene were applied to five sites of the abdominal skin. Dosage of each of the solutions was 0.01 ml. No irritation or other changes were noted. However, when 0.05 ml of the ten percent solution was applied to the back of a monkey for three consecutive days, the skin became severely irritated and necrotic. Subsequent experiments using more concentrated solutions (20, 40, 60 and 90 percent) were applied in the dosage of 0.05 ml on separate areas of the monkey's backs. At all concentrations, there was discoloration of the skin, ranging from very light to dark tan as the concentration increased. The discoloration was followed by swelling, which varied from slight to severe, again depending on concentration. The highest concentration caused cracking, oozing and serous discharge from the treated areas; intermediate concentrations produced hardening and swelling of the skin.

Among guinea pigs, the application of 0.01, 0.10 and 1.0 percent solutions caused no alterations of the skin, however, more concentrated solutions (40, 60 and 90 percent) resulted in discoloration, hardening and necrosis of the skin at the application site. Based on these tests, it appears that the threshold concentration at which hex in Ultrasene induces irritation of the intact skin lies between 10 and 20 percent for monkeys and between 1.0 and 40 percent for guinea pigs.

More recently, the irritant properties of hex were examined in a study conducted by the International Research and Development Corporation (IRDC, 1972). These tests were commissioned by Velsicol Chemical Corporation in accordance with the regulations of the Federal Hazardous Substances Act.

IRDC (1972) reported the results of an investigation of acute dermal toxicity of hex to rabbits. Four male and four female New Zealand white rabbits were used in this test. The hair was removed from the back of each rabbit with electric clippers. Two male and two female rabbits were used at each of two dosage levels. The test compound was applied in a single administration to the back of each rabbit at a dosage of 200 or 2000 mg/kg body weight. The area of application was wrapped with a gauze bandage and occluded with saran wrap. Twenty-four hours later, the bandages were removed and the backs were washed with water. The rabbits were observed for mortality for a period of 14 days.

All of the animals which received 2000 mg/kg dosage died within 24 hours after application of the compound. At the 200 mg/kg dosage, both male rabbits died but the female rabbits survived although both females exhibited weight loss over the 14 day period. The male rabbits that died showed weight loss also. In addition, cachexia, marked dermal irritation and hypoactivity were observed. Skin at the site of application turned purple in color within a few hours after hex application. Based on these results, hex was concluded to be "a highly toxic material by the dermal route of exposure" in accordance with the criteria established under the Federal Hazardous Substances Act.

Hex was tested for eye irritancy by instilling 0.1 ml of the "test compound" (which was presumably undiluted liquid hex) into the eyes of New Zealand White rabbits (IRDC, 1972). The test material was placed into the conjunctival sac of the right eye of each rabbit; the left eye served as an untreated control. Damage to the eye was evaluated by instillition of sodium fluorescein into the eye, followed by examination of the corneal surface for evidence of ocular damage under ultraviolet light. A graded scale was used to quantify the extent and severity of damage. The eyes of the rabbits were checked for corneal lesions at periodic intervals (at 1, 24, 48, 72 hours post exposure and at 7, 14, and 21 days post-exposure). Examinations at 14 and 21 days, however, were precluded by deaths of all of the rabbits on or before the 9th day of observation period.

Based on the severity of the ocular lesions produced in the rabbits, hex was concluded to be "an extreme irritant and probable corrosive substance" in the five minute test and "an extreme irritant and corrosive substance" in the 24 hour wash test (IRDC, 1972). These classifications are set in accordance with standards set under The Federal Hazardous Substances Act, specifically Part 191, Hazardous Substances Test for Eye Irritants, Food and Drug Administration.

4.1.3 Inhalation Tests

Treon, et al., (1955) exposed various animal species to vapors formed by bubbling a stream of air through liquid hex contained in a bubbling tower. This air was then mixed with clean air to achieve the desired concentration. The stream of air, conditioned with respect to temperature, dust content and humidity was then passed into a plywood exposure chamber in which the test animals were confined. A series of hex concentrations in the air in the exposure chamber were used; these varied from 0.0017 to 0.804 mg/l or 0.15 to 73.6 ppm, respectively. Test species were guinea pigs, rats, mice, and rabbits.

The authors reported that hex vapors were very toxic to all four species of animals. Exposure to the concentration of 13.0 ppm (an intermediate level in this experiment) for 15 minutes produced fatalities in all species except guinea pigs. Of the 4 species, rabbits appeared to be the most susceptible. Mice, rats and guinea pigs followed in order of decreasing susceptibility. Table 4.1 depicts the results of the inhalation experiments. The values tabulated correspond to the concentration in ppm which: (1) permitted all animals to survive; (2) killed some, but not all animals and (3) uniformly lethal conditions.

Animals of the following species died regularly when exposed to hex vapors at the following concentrations and durations: rabbits - 1.5 ppm for 7 hours; mice - 1.4 ppm for 2 seven-hour periods; rats - 1.0 ppm for 5 seven-hour periods or 3.2 ppm for 2 seven-hour periods and guinea pigs - 3.2 ppm for 2 seven-hour periods.

When mice, rats, rabbits and guinea pigs were exposed to 0.34 ppm in air for seven hours a day for 5 days per week, none of the mice or rats survived more than 20 such exposures. Two thirds of the rabbits had died by the end of the 25th period, however the guinea pigs survived through 30 periods. At 0.15 ppm, some animals from all four species survived 150 seven hour exposures over a period of 216 days. Eight percent of the mice did not survive the prolonged intermittent exposure. Details of these findings are discussed under the heading "chronic toxicity" in the next section.

IRDC (1972) also reported the results of acute inhalation experiments in rats. The test animals were exposed to atmospheric concentration of approximately 2 and 200 mg/l of the test compound for 4 hours. Due to the extremely high dosages employed (176.2 and 17624 ppm, respectively) little information could be derived from the study. No justification of the choice of dosages was given. All of the animals receiving the test compound at either exposure level died within 48 hours. All rats at the 200 mg/l dosage level died during the four hour exposure period. At the 2 mg/l atmospheric concentration 1 rat died during the exposure period, 8 were dead within 48 hours and 1 died on the second day of observation.

Signs seen during the exposure period included eye squint, dyspnea, cyanosis, salivation, lacrimation and nasal discharge. Gross necropsy showed gray coloration of the skin, severe hemorrhage of the lungs and hydrothorax among rats exposed to 200 mg/l. Rats exposed to 2 mg/l revealed congestion of the lungs in all cases.

Based on these results the investigators concluded that hex is a highly toxic material by the inhalation route of administration.

4.2 CHRONIC TOXICITY

4.2.1 Oral

In Treon, et al's., (1955) study, rabbits and rats given various dosages of hex ranging from 180-2100 mg/kg tended not to survive long enough at these dosages to provide acceptable data on chronic oral toxicity. Individuals which survived and were killed subsequent to

TABLE 4.1. DOSE RESPONSE DATA: INHALATION OF HEX VAPORS[a] FOR VARYING EXPOSURE TIMES

Species of Animal	Fatalities, Percent	Hex Concentration, ppm 1.0 hr	3.5 hr	7.0 hr
Guinea pigs	0	7.2	3.1	1.5
	50	13.8	7.1	3.2
	100	20.0[b]	12.4	6.7
Rats	0	3.1	1.4	1.5[c]
	50	7.2	3.1	3.2[d]
	100	20.0[b]	7.1	6.7
Mice	0	1.4	1.4[b]	--
	40	7.2	3.1[e]	1.5[e]
	100	13.8	7.1	3.2
Rabbits	0	1.4	--	--
	67	3.1	6.4	--
	100	7.2	7.1	7.5

[a] Source: Treon,et al., 1955.

[b] Duration of exposure was 1.25 hr.

[c] 25% of group died.

[d] 75% of group died.

[e] 80% of group died.

[f] 20% of group died.

exposure showed degenerative changes in the liver, kidney tubules, brain and adrenal glands. These effects appeared to be at least partially reversible in that the severity of these lesions diminished as the interval of survival lengthened (after cessation of oral hex administration). Symptoms and pathological changes exhibited in these animals are more fully described under the topic of effects. In any event, Treon, et al., did not establish an oral dosage which could be tolerated (e.g. without mortality) over an extended period of time.

Studies in the Soviet Union reported by Naishstein and Lisovskaya (1965), appear to provide the only source of information on the effects of long-term, low-dose exposure to hex. Investigations of three aspects of hex toxicity were reported: (1) minimum lethal dose and cumulative effects; (2) dermal toxicity and (3) effects of prolonged ingestion of low doses of hex. Naishstein and Lisovskaya found the minimum lethal dose of hex for white rats was 600 mg/kg body weight. Note that this value is equivalent to the upper range of LD_{50}'s (420-620 mg/kg) reported by Treon, et al., (1955) and by IRDC (530-630 mg/kg). No explanation for this discrepancy is given, however, it should be noted that no assay of the material designated C-56 was reported by these investigators. Also, although it was stated that hex was given in oil solution, the exact method of administration (e.g. mixing with food, intubation, etc.) could not be determined from the report. Daily administration of 1/30 of the minimum lethal dose (20 mg/kg) for 6 months killed only 2 animals out of 10, even though the cumulative dose received was 1.5 times the LD_{100}, or uniformly lethal dose. Although some changes were noted in the weight coefficients of the internal organs of the animals, the authors judged the cumulative effects of hex to be weak.

4.2.2 Dermal

Treon, et al., (1955) reported that dosages of less than 10 percent hex appeared to be tolerated without irritative effects in monkeys and probably also in guinea pigs. Unfortunately, the authors did not continue the low dose regimen for a sufficient period to observe chronic effects. High concentrations, 430-6130 mg/kg, applied to the skin of rabbits were frequently fatal within a few hours. Six rabbits which survived for 7-21 days after application of hex were killed and autopsied. Degenerative changes were seen in the brain, liver, kidneys and adrenal glands of these animals in addition to chronic skin inflammation, acanthosis; hyperceratosis and epilation. Visceral lesions due to dermal hex application reported by Treon, et al., (1955) are described in the section on pathological effects.

Naishstein and Lisovskaya also investigated the effects of prolonged, low-dose dermal exposure to hex. These experiments consisted of applying 0.5-0.6 ml of a concentration of 20 ppm hex in aqueous solution to the shaved skin of rabbits daily for a period of 10 days. No differences were detected between the skin of the experimental animals and that of the control animals.

According to Naishstein and Lisovskaya (1965), papers by Soviet and other researchers have demonstrated the important part played by the hypopysis-suprarenal cortex system in nonspecific reactions of the organism to unfavorable factors. Spynu (1959) in particular, in his studies on the functional state of this system when affected by chemical agents of low intensity (including poisonous chemicals in diene synthesis), noted changes in weight and ascorbic acid content of the suprarenals. Naishstein and Lisovskaya failed to find significant differences between exposed and unexposed rabbits with respect to these parameters.

4.2.3 Inhalation

Guinea pigs exposed to a concentration of 0.34 ppm hex for seven hours per day, five days a week survived until they reached 30 periods of exposure (6 weeks). However, rats and mice exposed to this concentration survived only 5 periods of exposure. Survival of the rabbits was intermediate; 2/3 had died before the end of the fifth week (25 exposure periods).

A lower concentration, 0.15 ppm hex, was tolerated by guinea pigs, rabbits and rats throughout 150 seven-hour periods of exposure extending over a period of approximately 7 months. Four of five mice died within this period. The guinea pigs, rabbits and rats grew normally during this period, however, slight degenerative changes were seen in the livers and kidneys of these animals. These changes are discussed under the heading, "Pathological Effects."

At the relatively high concentrations, many of the animals died during the exposure period whereas with the lower exposure levels most of the animals died days or weeks after the cessation of exposure.

4.3 SYMPTOMS AND PATHOLOGICAL EFFECTS

4.3.1 Oral Administration

Signs of intoxication in rabbits and rats dosed orally with hex in the Treon, et al., (1955) acute toxicity studies included diarrhea, lethargy, and retarded respiration rate. The odor of the compound could also be detected in the feces of the animals and on their bodies, presumably from fecal contamination.

Among the rabbits who died, diffuse degenerative changes were seen in the brain, heart, liver and adrenal glands. Degenerative changes were also seen in the epithelium of the renal tubules and the lungs of these animals were congested and edematous. The same types of degenerative changes were also noted in the rats. In addition, some of the rats showed acute necrotic gastritis in the stomach. Interestingly, those animals which survived the oral tests and were sacrificed some time later exhibited residual degenerative changes of the type described above. This suggests that the pathological changes are semi-permanent; the severity of the lesions did diminish as the length of the post-exposure survival interval increased, however.

Hooker Chemicals and Plastics Corporation commissioned a series of toxicologic studies of hex. This work, performed by Industrial Bio-Test Laboratories (IBT) consisted of 4 separate investigations: (1) a 90-day oral toxicity study (rats); (2) a 28-day subacute dermal toxicity study (rabbits); (3) an acute vapor inhalation study (rats); and (4) a 28-day subacute vapor inhalation study. Since these studies focused primarily on symptoms and toxic manifestations/effects, (rather than establishing or documenting toxic levels) they are reported in this section.

It should be noted that we were unable to obtain the original reports of IBT's test results, and the following summary is based on information contained in a review document on hex prepared by Equitable Environmental Health (1976) under contract to Hooker Chemical and Plastics Corporation, rather than the actual IBT test results. While we believe this information to be accurate, we do not assume responsibility for any errors which may have been committed by EEH in interpreting the results of the IBT results. As reported by EEH, IBT conducted a 90-day subacute oral toxicity study, again using albino rats. Hex was mixed into the animals food at concentrations of 0, 30, 100 or 300 ppm. No effects were seen in any of the parameters measured: growth, food intake, mortality, abnormal behavior, hematology, clinical blood studies, and urinalysis. Gross pathologic examination also failed to reveal any abnormalities which could be attributed to ingestion of hex. Similarly, organ weights and ratios and microscopic examination of tissues and organs failed to show treatment - related abnormalities.

Naishstein and Lisovskaya carried out another chronic toxicity experiment on 90 white rats weighing 100-120 grams. The dosages employed were 0.002, 0.0002, and 0.00002 mg/kg (0.04, 0.004 and 0.0004 mg/liter). The first dose was 30 times greater than the threshold concentration with respect to aftertaste and smell; the second dose corresponded to the practical limit of detection by smell and the third dose was 10 percent of the second. No deviations were observed in the behavior of the rats or in their weights throughout the 6 month experimental period. Likewise, no significant changes in hemoglobin, red blood cells, white blood cells or peripheral reticulocyte counts in the experimental group as opposed to the controls. Among animals receiving the highest dose, 0.002 mg/kg, neutropenia and a tendency toward lymphocytosis were noted. Peripheral blood studies of animals at the lower levels showed no abnormalities however.

Other parameters, including tests for behavioral alterations (testing of conditioned reflexes) were studied, however, no conclusions were drawn from this data as it was considered by the investigators to be unreliable.

4.3.2 Dermal Application

Treon, et al., (1955) showed that dermal application of very low dosages of hex (0.25 ml/kg) were extremely irritating and induced local discoloration and edema. The skin became hard, encrusted and fissured several days after application. The extent of the local damage varied directly with the size of the dose applied. At autopsy rabbits exhibited visceral lesions similar in appearance to those seen after oral administration of hex. Again, diffuse degenerative changes were seen in the brain, heart, adrenals, liver cells, and kidney tubules. Pulmonary hyperemia and edema were also noticed. Animals killed 7-21 days post-application of the compound showed evidence of the same type of degenerative changes.

Monkeys dosed with various concentrations of hex in solution exhibited discoloration of the skin which increased directly as the concentration of hex applied increased. Swelling, oozing and encrustation similar to that described above for rabbits was seen. Eventually healing took place, but scarring and hair loss in the area of application appeared to be permanent.

IBT also reported results of a 28-day subacute dermal toxicity study using albino rabbits. (EEH, 1976). Hex solution was allowed to contact the shaved, unoccluded skin of rabbits for an unlimited period of time (test material not washed off). The test animals were dosed 5 days a week for a period of 4 weeks, or 20 applications in all. The concentration of hex in Group I was 0.1 per cent (weight/volume); in Group II, the concentration was 0.5 per cent. None of the animals died and no pharmacotoxic symptoms were noted, however, both hex solutions were extremely irritating to the skin and slight losses in body weight occurred in some of the rabbits receiving the higher concentration (Group II). No adverse effects were noted in hematological studies, clinical blood chemistry studies, and urinalysis. No significant gross or microscopic pathology was noted, except of course, the local skin, lesions. Gross skin changes were characterized by fibrosis, escharosis (scarring) and slight-to-severe desquamation. Microscopic examination revealed acanthosis and hyperkeratosis involving the epidermis. This effect was seen in a few of the animals in Group I and most of the animals in Group II. Such findings were attributable to the irritant action of the test compound.

4.3.3 Inhalation Tests

Animals who survived the vapor exposure sessions lost weight and many of these animals still had not regained their initial weights as long as 6 to 8 weeks after inhalation of hex.

Animals (rats, rabbits, guinea pigs and mice) exposed to vapors of hex showed signs of extreme irritation of the eyes and mucous membranes (Treon, et al., 1955). At very high concentrations (46.5 ppm) animals responded by rubbing their noses with their forefeet, closing their eyes and retracting their heads. This was accompanied by sneezing, tearing and irregular breathing. In less than 30-60 minutes the animals were gasping for breath.

Lower concentrations of hex vapor (12.4 and 13.8 ppm) produced similar irritation of the mucous membranes although somewhat milder in degree. The same symptoms were even seen at the very low dosages 1.0 and 1.6 ppm, however, the symptoms developed over a period of hours, rather than minutes. Exposure to very low concentrations (0.33 ppm and 0.15 ppm) resulted in some irritation of the eyelids and increased respiratory rate, however, in the case of the latter dosage (0.15 pm) irritation was only in the mice, which developed mild respiratory changes (Treon, et al., 1955).

At autopsy, degenerative changes similar to those described from oral and dermal administration were seen in all species of animals tested. Prolonged intermittent exposure to as little as 0.15 ppm hex vapor concentrations induced slight degenerative changes in the livers and kidneys in all species of animals employed.

Industrial Bio-Test Labortories (IBT) also conducted two vapor toxicity studies: an acute test and a 28-day subacute test (EEH, 1976). In the acute vapor toxicity tests, Charles River rats were exposed for 4 hours to varying concentrations of hex in air. An acute LC_{50} of 3.67 ppm (0.041 mg/l of air) was reported. Complete necropsies were performed at death for those who died shortly after exposure and at the termination of the study for those who survived. Acute pneumonia was observed in the rats which died following exposure to the test material. Treated rats that survived to the end of the study showed emaciation and chronic proliferative inflammatory changes in the lungs.

The second IBT test consisted of a 28-day subacute vapor inhalation study using albino rats. Two groups of 10 rats each were exposed to hex vapor for 6 hours per day, 5 days per week for 4 weeks. A third group of ten rats served as untreated controls. One group (Group I) was exposed to vapor containing 0.529 ppm hex (0.006 mg/l) and the other experimental group. (Group II) was exposed to 1.23 ppm (0.014 mg/l). Hematologic and clinical chemistry studies and urinalysis were performed on days 0 and 28. On the 28th day the animals were sacrificed. EEH made no mention of mortality in either exposure group, so presumably all animals survived until the time of sacrifice. Neither hematologic nor clinical studies or urinalysis revealed any abnormalities directly attributed to the test material vapor. Statistical analysis did reveal increases in absolute liver weight and liver: brain weight ratios among the rats exposed to 1.23 ppm hex vapor. Gross pathological examinations failed to reveal abnormalities attributable to the test compound; microscopic examination of tissue revealed hepatocytomegaly and necrotizing hepatitis. These effects were thought to be attributable to hex exposure.

An early study by Ingle (1953) showed that previous observations of vapor toxicity of chlordane could be explained on the basis of unreacted ingredients in earlier chlordane formulations. Chief among these unreacted materials was hex, which caused samples of chlordane to give off irritating volatiles. This problem had been eliminated by 1953 by the more complete removal of unreacted ingredients in the pesticide. Ingle (1953) tested the hypothesis that hex may have been the actual agent responsible for high rates of mortality among mice in previous chlordane tests by replicating an earlier study using relatively pure chlordane and chlordane mixed with hex in varying (2.5-10.0 percent) proportions. The actual concentration of hex in the exposure chamber was not reported. Albino mice exposed to air passing through a saturation train containing the test solutions exhibited the same toxic symptoms and high mortality with the exception of the "control" group exposed only to chlordane. Onset and severity of symptoms were directly proportional to the volume of added hex.

Gross pathologic findings in the organs of the mice included hemorrhagic areas in the lungs and lesions of unspecified type in the liver. Microscopic findings included congestion of capillaries and edema of the lungs, coagulative necrosis, hyalinization, bile duct proliferation and cytoplasmic oxyphilia. Kidney damage included protein leakage, degeneration of the tubular epithelium and capillary engorgement in the glomerular tufts. Extent of tissue injury was proportional to the volume of added hex. Thus, Ingle concluded that previously reported vapor toxicity to mice should not have been attributed to chlordane, but rather to an unreacted intermediate, namely hex.

4.4 COMPARATIVE TOXICITY

Treon, et al., (1955) found hex to be more toxic than either phosgene or carbon tetrachloride. Based on acute vapor toxicity to rabbits, hex was found to be considerably more toxic, based on comparable atmospheric concentration. Whereas less than 1/2 of a group of rabbits died following exposure to 75-80 ppm phosgene for 30 minutes, exposure of rabbits to 7.2 ppm hex produced death in 1/3 of the test animals. Exposure to the same concentration for 60 minutes was uniformly lethal.

On the basis of actual toxicity, hex in the concentration of 0.15 ppm is roughly comparable to carbon tetrachloride at 100 ppm.

4.5 METABOLISM

Only two studies which address the metabolism of hex could be located (Mehendale, 1977; Kommineni, 1978). The latter study focuses upon absorption and elimination of hex while the Mehendale study is more concerned with the disposition of hex within the body and modes of elimination.

The Kommineni study consisted of two parts. The first consisted of a study of rats exposed to various doses of hex by gavage while the second portion examined guniea pigs exposed to varying doses of hex via dermal application. Inferences regarding patterns of absorption, metabolism, and excretion are based on gross pathology findings and histopathologic findings at necropsy.

In the first series, a total of 10 female rats were exposed to 0, 50, 100, 150, 200, and 300 mg/kg of hex by gavage. All animals were sacrificed 24 hours post-treatment. The rats were necropised and lungs, liver, spleen, kidneys, adrenals, heart, stomach, and intestines were saved for histopathology evaluation.

Gross pathology of the rats exposed to 200 and 300 mg/kg revealed brown discoloration around the nostrils and anus of the rats. The urinary bladders of two of the four rats contained brown fluid. Subserosal emphysema of the nonglandular stomach was evident in one animal. On histopathologic examination, the lungs showed atelectasis with moderate thickening of the alveolar walls. The alveolar walls contained moderate numbers of macrophages and neutrophils. Some bronchi contained denuded epithelium. No edema was present in the lungs. Rats receiving lower dosages showed similar, but milder, changes. The stomachs of rats receiving dosages of 200 or 300 mg/kg showed coagulative necrosis of the gastric squamous epithelium. The submucosa of the nonglandular part of the stomach (submucosa, submuscularis, muscularis) showed moderate edema. Epithelium of the glandular part of the stomach showed no treatment-related changes. Animals receiving lower doses showed similar changes in the stomach. Ulcers of the nonglandular portion of the stomach were seen in several of the animals. At all dosages, the other organs were unremarkable.

The author commented that these morphological changes indicate that hex is absorbed through the squamous epithelium of the nonglandular part of the stomach and that the major route of elimination of hex is through the lungs.

In the second part of the study, four male guinea pigs were painted on the skin (site unspecified) with hex at dosages of 0, 300, 600, and 1200 mg/kg and sacrificed 24 hours after the exposure. All animals were necropsied and the lungs, liver, pancreas, kidneys, adrenals, urinary baldder, heart, skin, stomach, and intestines were saved for histopathologic evaluation.

On gross pathology, subcutaneous edema was seen extending from the inguinal area to the sternum. At the lowest dosage, the lungs were highly expanded and showed rib impressions on the parietal surface. Similar but more severe changes were seen in the animal receiving 600 mg/kg. The animal painted with 1200 mg/kg expired prior to sacrifice; the trachea was filled with frothy fluid. Histopathologic examination of the lungs revealed atelectasis with thickened alveolar walls containing moderate numbers of macrophages and neutrophils. Intense congestion of all pulmonary blood vessels and occasional alveolar edema was seen in the animal receiving the 1200 mg/kg dose. In the skin, moderate to marked edema disrupted the collagen bundles. Focal pockets of neutrophils were seen in the edematous dermis. Edema extended throughout the thickness of the adipose tissue layer. One animal showed partial thrombosis of medium size veing situated deep in the dermis. The skin appendages were normal.

Kommineni concluded, "Hex is absorbed through the skin and probably is eliminated through the lungs. Unlike the rat stomach, the squamous epithelium of the guinea pig skin and its adnexa did not show necrotic changes. This is probably due to two factors, surface area and transit time".

In the Mehendale (1977) study, radiolabeled hex was administered by oral intubation to four male Sprague-Dawley rats in order to examine absorption, metabolism, and excretion of the compound following a single oral dose. After dosing with ^{14}C-hex (5 u moles, 1 uCi per animal), the rats were maintained in metabolism cages for 7 days, during which daily urine and fecal samples were collected. After 7 days, the animals were sacrificed and the major organs were removed and radioassayed.

Urine and powdered fecal samples were radioassayed for total ^{14}C. An average of approximately 33 percent of the total dose was excreted in the urine after 7 days. About 87 percent of that (approximately 28.7 percent of total dose) was eliminated during the first 24 hours after the administration of the compound. Fecal excretion accounted for 10 percent of the total dose; nearly 60 percent of the 7-day fecal excretion occurred during the first day.

Beyond the third day after treatment, only trace amounts of the hex-derived ^{14}C were eliminated in the feces. Tissues retained only trace amounts of hex after 7 days. For example, the kidney retained only about 0.5 percent of the total dose and the liver less than 0.5 percent. Other organs and tissues--fat, lung, muscle, blood, etc.--contained even less of the radiolabel. Such findings suggest that at least half of the administered hex was eliminated by routes other than urine and feces. The author felt that the respiratory tract is probably the major route of excretion.

The nature of the radioactivity excreted in the urine was examined searching for possible metabolites. it was found that about 70 percent of the radioactivity in the urine was extractable using a hexane:isopropanol (9:1) mixture. The organic solvent was concentrated, applied to thin-layer chromatography (TLC) plates, and developed in three solvent systems. The radioactive spots were visualized by auto-radiography on medical x-ray film. The results suggested the presence of at least four metabolites; however, at the time of this writing they had not been identified and characterized.

Disposition and biliary excretion of ^{14}C-hex was studied by injection of approximately 1 uCi (5 u mole) of ^{14}C-hex into the femoral vein of anesthetized rats. Timed samples of blood and bile were collected for 1 hour from the femoral artery and common bile duct which had been cannulated prior to dosing. Approximately 9 percent of the administered dose was excreted in the bile in 1 hour. Because this quantity is equivalent to that excreted in the feces over 7 days, enterohepatic circulation of this compound is probable. The nature of the compound present in the bile is not yet known.

At the end of the above experiments, the animals were sacrificed and the liver and kidneys were removed. Tissue homogenates from the organs were radioassayed and the distribution of the radioactivity among the various subcellular fractions was examined by assaying the various centifugation fractions. Kidney cytosol accounted for 93 percent of the radioactivity in the total kidney homogenate. This behavior is consistent with rapid urinary excretion. Similary, 68 percent of the radioactivity in the liver homogenate was associated with the liver cytosol fraction, once again consistent with rapid excretion.

Pre-exposure of some of the rats to hex (50 mg/kg/day) for 3 days prior to the experiment did not affect blood decay curves and biliary excretion; however, an increased concentration in the kidneys after a single challenge with ^{14}C-hex was observed.

Whitacre (1978) reported that Vesicol has contracted an independent metabolism study in rats and mice using ^{14}C-hex. The metabolism of hex was determined both after single acute dosing and repeated administration over a period of about 30 days. The results of these studies have not yet been officially reported although verbal appraisal of some results has been provided to Velsicol.

It appears that results of this study do not agree closely with the Mehendale study. The recent study shows hex to be eliminated from mammals (mice and rats) mainly by the fecal route and with no more than about 15 percent being eliminated in urine. Further, these studies do not indicate any significant amounts of pulmonary elimination of hex or its metabolites. Whitacre (1978) believes that the poor recoveries in feces in the Mehendale study may be the result of volatility of hex or its metabolites before removal for analysis. Losses during sample preparation undoubtedly further complicate the analysis of fecal matter.

4.6 Teratogenicity

International Research and Development Corporation (1978) has recently completed a pilot teratology study using pregnant Charles River (CD) rats. Negative findings with respect to teratogenic effects were reported for oral hex dosages up to 100 mg/kg/day.

The test protocol employed in the pilot teratology study involved administration of various dosages of hex to 30 female Charles River (CD) rats approximately 12 weeks of age. Females were mated with male rats of the same strain. After mating, the females were assigned to six groups, one control and five treatment groups of five rats each. Hex was dissolved in corn oil and administered by gavage from day 6 through day 15 of gestation. Dosage levels of 3, 10, 30, 100, and 300 mg/kg/day were administered to the test groups and the control group was given the vehicle (corn oil) on a comparable regimen of 10 ml/kg/day.

During gestation, the females were observed for clinical signs of toxicity, mortality, and body weight gains. They were then sacrificed on gestation day 20 and the uterine contents examined for viable and nonviable fetuses, early and late resorptions, and total implantations. There were no differences in the four treatment groups given 100 mg/kg/day or less when compared to the control group in terms of number of viable or nonviable fetuses, resorptions, implantations, or corpora lutea. Rats receiving doses of 3 or 10 mg/kg/day showed no treatment-related changes in appearance or behavior. Rats receiving 30 mg/kg/day or higher showed staining of the anogenital area and reduced body weight gains. The females in the 100 mg/kg/day group had body weight losses during the first 3 days of treatment and reduced weight gains for the remainder of the study. Survival was 100 percent for all rats given 100 mg/kg/day or less. All rats in the 300 mg/kg/day group were dead by gestation day 10.

Various reproductive parameters examined in the pilot teratology study are shown in Table 4.2

4.7 Mutagenicity

Hex has been tested for mutagenicity and reported non-mutagenic in both short-term *in vitro* mutagenic assays (National Cancer Institute, 1977; Industrial Bio-Test Laboratories, 1977; Litton Bionetics, 1978a) and in a mouse dominant lethal study (Litton Bionetics, 1978b).

The National Cancer Institute (1977) reported that preliminary results indicated that hex was non-mutagenic in *Escherichia coli* K12 (mutation site not specified) in the presence of a mammalian metabolic activation system containing mouse liver microsomes.

Negative results were also reported by Industrial Bio-Test Laboratories (1977) using a test protocol almost identical to the Ames Mutagenic Assay (Ames, et al. 1975). The tests used four or five strains of *Salmonella typhimurium* with and without metabolic activation. Hex was dissolved in acetone and added to the microbial assay plates in dosages from 10-5000 ug/10 ul. Concentrations greater than 10 ug/10 ul produced a bacteriocidal effect in three of the strains tested; a posible lethal effect occurred at 2500 ug/10 ul or greater in the fourth strain. A repressive effect was noted in three of the strains at concentrations below 10 ug/10 ul. Volatilate (volatile vapors) of hex were also tested on one strain using the vapor from hex concentrations of up to 2500 ug/10 ul and exposure times of up to 2 hours. Results from two successive assays in the absence of rat liver enzymes (hex concentrations 10, 25, 50, 75, and 100 ug/10 ul) were negative in all four tester strains. Two assays using the same dosages in the presence of rat obtained for the hex effusate as well. The investigators expressed concern over the repressive effective of hex on the test bacteria, stating "It appears that hex is

TABLE 4.2. PILOT TERATOLOGY STUDY IN RATS: CAESAREAN SECTION DATA FOR INDIVIDUAL FEMALES.[a]

Dosage Level Dam Number	Viable Fetuses	Nonviable Fetuses	Late Resorptions	Early Resorptions	Plant Implantation Loss	Implan- tations	Corpora Lutea
Control:							
73662	13	0	0	1	1	14	14
77334	14	0	0	1	1	15	15
77336	12	0	0	1	1	13	13
77428	11	0	0	1	1	12	22
77428	15	0	0	0	0	15	16
Total	65	0	0	4	4	69	80
Mean	13.0	0.0	0.0	0.8	0.8	13.8	16.0
3 mg/kg/day:							
73642	16	0	0	0	0	16	16
77342	17	0	0	0	0	17	17
77343	16	0	0	0	0	16	16
77426	12	0	0	1	1	13	18
77428	15	0	0	0	0	15	15
Total	76	0	0	1	1	77	82
Mean	15.2	0.0	0.0	0.2	0.2	15.4	16.4
10 mg/kg/day:							
77304	17	0	0	0	0	17	18
77309	13	0	0	0	0	13	13
77346	11	0	0	3	3	14	14
77427	12	0	0	0	0	12	13
77436	15	0	0	0	0	15	15
Total	68	0	0	3	3	71	73
Mean	13.6	0.0	0.0	0.6	0.6	14.2	14.6
30 mg/kg/day:							
77310	14	0	0	0	0	14	14
77313	13	0	0	1	1	14	16
77350	6	0	0	0	0	6	7
77438	11	0	0	0	0	11	14
77450	12	0	0	0	0	12	14
Total	56	0	0	1	1	57	65
Mean	11.2	0.0	0.0	0.2	0.2	11.4	13.0
100 mg/kg/day:							
73673	16	0	0	0	0	16	16
77302	14	0	0	0	0	14	14
77314	15	0	0	2	2	17	17
77415	11	0	0	0	0	11	11
77439	12	0	0	0	0	12	12
Total	68	0	0	2	2	70	70
Mean	13.6	0.0	0.0	0.4	0.4	14.0	14.0
300 mg/kg/day:							
73758	Died, gestation day 9 - gravid						
77324	Died, gestation day 10 - gravid						
77333	Died, gestation day 10 - gravid						
77417	Died, gestation day 10 - gravid						
77445	Died, gestation day 10 - gravid						

[a] Source: International Research and Development Corp. (1978).

probably non-mutagenic and that some toxic effect prevailed with respect to the tester strains required for this assay. Analysis of variance and multiple comparison of the data confirms this observation".

Litton Bionetics (1978a) conducted a mouse lymphoma cell assay in order to evaluate the capability of hex in inducing specific locus forward mutation. The indicator cells used in the assay were Fischer mouse lymphoma cells derived from cell line L5178Y. These cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine (BUdR) sensitive. Scoring for mutation is based on selecting cells which have undergone forward mutation from a TK+/-to a TK-/- genotype by cloning them in soft agar with BUdR. Cells were maintained in Fischer's medium for leukemic mouse cells with 10 percent horse serum and sodium pyruvate. The dosages used in the test were predetermined by exposing the cells to a wide range of hex concentrations and measuring the reduction of growth potential following a 4-hour exposure at each dose. The maximum dose selected was that which produced a 50 percent reduction in growth. The actual hex dosages employed were: 0.00040, 0.00048, 0.00056, 0.00064, and 0.00125 ul/ml in the activated series (mouse liver microsomes were added to the growth medium). A nonactivated series using somewhat lower dosages was included also.

Both negative and positive controls were used; the negative control for both series was the solvent dimethylsulfoxide (DMSO), whereas ethylmethanesulfonate (EMS) and dimethylnitrosamine (DMN) were used as positive controls in the nonactivated and activated systems, respectively. Hex was added to the cells in the growth medium for 4 hours. The cells were then washed and allowed to express in the growth medium for 3 days. After the expression period, results were evaluated by counting the TK-/-mutants after cloning the cells in a selection medium (soft agar with BUdR).

Hex dissolved in DMSO was evaluated over the concentration range of 0.0000025 ul/ml to 0.00125 ul/ml. Considerable toxicity occurred at concentrations greater than this and the extent varied according to the presence of the mouse liver activation system as shown in Table 4.3. No cells treated with hex (at the concentrations shown) survived in the non-activated system.

Hexachlorocyclopentadiene did not induce forward mutation in L5178Y cells. The data presented in Table 4.3 show the concentrations of the test compound employed, the number of mutant clones obtained, surviving populations after the expression period, and calculated mutation frequencies. No dose-related trends in either absolute number of mutants or mutant frequencies were observed, and at no level did any of the test parameters increase significantly over the spontaneous level. Consequently, hex was considered to be nonmutagenic under the conditions of this assay.

TABLE 4.3. SUMMARY OF MOUSE LYMPHOMA (L5178Y) RESULTS.[a,b]

TEST[c]	S-9		Daily Counts (Cells/ml x 10ES)			Relative Suspension Growth (% of Control)	Total Mutant Clones	Total Viable Clones	Relative Cloning Efficiency (% of control)	Percent Relative Growth[d]	Mutant Frequency X 10E-6[e]
	Source	Tissue	1	2	3						
NONACTIVATION											
Solvent Control	--	--	16.8	10.2	13.8	100.0	48.0	257.0	100.0	100.0	18.7
Negative Control	--	--	13.2	12.0	15.0	100.5	48.0	234.0	91.1	91.5	20.5
EMS[f] 0.5 µl/ml	--	--	9.0	9.2	11.8	41.3	597.0	89.0	34.6	14.3	670.8
ACTIVATION											
Solvent Control	Mouse	Liver	15.2	9.6	13.2	100.0	55.0	281.0	100.0	100.0	19.6
Negative Control	Mouse	Liver	14.2	13.0	10.6	101.6	39.0	293.0	104.3	105.9	13.3
DMN[g] 0.5 µl/ml	Mouse	Liver	7.2	7.6	8.2	23.3	322.0	55.0	19.6	4.6	585.5
Test Compound											
0.00002 µl/ml	Mouse	Liver	16.8	9.0	10.6	83.2	99.0	288.0	102.5	85.3	34.4
0.00004 µl/ml	Mouse	Liver	13.0	12.4	9.6	80.3	50.0	269.0	95.7	76.9	18.6
0.00008 µl/ml	Mouse	Liver	12.4	9.8	16.2	102.2	55.0	194.0	69.0	70.6	28.4
0.00016 µl/ml	Mouse	Liver	13.6	13.8	7.4	72.1	45.0	359.0	127.8	92.1	12.5
0.00032 µl/ml	Mouse	Liver	18.2	9.0	10.0	85.0	38.0	309.0	110.0	93.5	12.3

[a] Source: Litton Bionetics, Inc. (1978a).

[b] Hexachlorocyclopentadiene dissolved in dimethyl sulfoxide.

[c] Concentrations are given in microliters (µl) per milliliter.

[d] Relative suspension growth x relative cloning efficiency/100.

[e] Mutant clones/viable clones x $10E^{-6}$.

[f] Ethylmethanesulfonate.

[g] Dimethylnitrosamine.

The mutagenic properties of hex were also evaluated in a dominant lethal study of mice (Litton Bionetics, 1978b). The dominant lethal assay provides a means of determining whether a compound is capable of inducing damage in the germ cells of treated male mice. Dominant lethality is manifested in various forms of fetal wastage, both pre- and post-implantation. Positive dominant lethal assays indicate that a compound is able to reach the developing germ cells. Chromosome aberrations including breaks, rearrangements, and deletions as well as ploidy changes and nondisjunction are believed to produce positive results on this test. Since substances capable of producing gross chromosomal lesions are probably capable of producing more subtle balanced lesions or specific locus mutations, the test also provides suggestive evidence of nonlethal mutations transmissible to future generations as well.

Litton Bionetics reported negative results, that is, there was no evidence of significant dominant lethal activity by hex in mice. The test protocol called for the assignment of ten random bred male mice to one of five groups. Three test groups received hex dosages of 1.0, 0.3, and 0.1 mg/kg, respectively. These dosages were determined by deriving an LD5 level (1.0 mg/kg) and taking one-third and one-tenth of that dose. A fourth group received only the solvent and the fifth group served as a positive control. Hex was mixed in the feed of the three experimental groups and the solvent control group for five consecutive days. The positive control group received a known mutagen, triethylenemelamine (TEM) in a single intraperitoneal injection. Two days following treatment, each male was caged withh two unexposed virgin females. At the end of seven days, these females were removed and replaced by two unexposed virgin females. This mating cycle was continued for seven weeks. Each pair of female mice was killed two weeks after mating and necropsied. Their uterine contents were examined for dead and living fetuses, resorption sites, and total implantations. All test parameters (fertility index, average implantations per pregnancy, average resorptions (dead implants) per pregnancy, proportion of females with one or more dead implantations, proportion of females with two or more dead implantations, and the ratio of dead implantations to total implantations were within normal limits based on historical concurrent control levels for this test. Thus, there was no evidence of dominant lethal activity in any of the hex treated groups. The positive control group, however, did show the expected dominant lethal activity.

4.8 CARCINOGENICITY

Various types of evidence may be used in evaluating the possible carcinogenic activity of a substance. In order of preference, these include: (1) human data; (2) animal data; (3) short-term (*in vitro*) tests; (4) metabolic pattern; and (5) structure-activity relationships. This section summarizes what is known about each of the above.

No epidemiologic studies or case reports examining the relationships between exposure to hex and cancer incidences could be found in the literature. Hooker Chemicals and Plastics Corporation reports that an in-house study of the mortality patterns of hex-exposed workers is now underway; however, the study is far from being completed (Zavon, 1978, personal communication). Other in-house studies of workers employed in the manufacture of pesticides (including hex) are reportedly being conducted by Velsicol Chemical Corporation. We were unable to obtain any further information on the current status or findings of these studies.

The National Cancer Institute concluded that toxicologic studies of hex in animals have not been adequate for evaluation for carcinogenicity (National Cancer Inst., 1977a). Chronic toxicity studies reported were based on too few animals in some cases and/or the duration of the experiments was too short for appropriate evaluation of chronic effects, including carcinogencity (World Health Organization, 1976; National Cancer Institute, 1969).

Only one short term *in vitro* test of hex for carcinogenic activity could be identified.

Litton Bionetics (1977) reported the results of a test to determine whether hex could induce malignant transformation in BALB/3T3 cells *in vitro*. The cells and methodology of the test were those of Kakunaga (1973). The basic rationale of the test and its validity as an indicator of carcinogenic activity was described by the investigators as follows:

> The endpoint of carcinogenic activity is determined by the presence of fibroblastic-like colonies which are altered morphologically in comparison to the cells observed in normal cultures. These (transformed) cells grow in criss-cross, randomly oriented fashion with overlapping at the periphery of the colony. The colony exhibits dense piling up of cells. On staining, the foci are deeply stained and the cells are basophilic in character and variable in size. These changes are not observed in normal cultures, which stain uniformly.

Cell cultures with very little or no spontaneous transformation are maintained for use in these tests. The data generated at each dose level of the test material are analyzed using the t statistic. A significant set of data for any dose level may be sufficient to indicate a positive response. Because this assay is still nonroutine, and definitive criteria for evaluation have yet to be developed, scientific judgement and expert consultation are needed for appropriate interpretation of results.

The BALB/3T3 cells used in the test were grown in Eagle's minimal essential medium (EMEM) supplemented by 10 percent fetal calf serum. Cultures were passaged weekly in 60 mm culture dishes. Approsimately 10,000 cells were seeded into 50 ml sterile tissue cuture flasks and incubated in EMEM to permit attachment. After the cells were attached, the control and test compounds were added to the plates. Dosages of 0.00001, 0.00002, 0.000039, 0.000078, and 0.000156 ul/ml of hex were employed. The maximal dosage, 0.000156 ul/ml, was determined by selecting from preliminary cytotoxicity tests the maximum dosage which permitted survival of at least 80 percent of the cells. 3-methylcholanthrene at 5 ug/ml was used as a positive control and the test compound solvent was used as a negative control. Ten replicates per dose level were prepared and chemical exposure was maintained for 48 hours. Plates were then washed free of the compound and replenished with fresh growth medium. The plates were then incubated for an additional 3-4 weeks with twice-weekly medium changes. Cell integrity was monitored by daily observations. Cells were separated from the medium, washed with saline, and stained. They were examined for stained foci; all potential foci were examined microscopically. Results were presented as the number of foci per set of replicate plates at each dosage level.

The test material was quite toxic to cells as indicated in the preliminary range-finding tests. No significant carcinogenic activity for hex was reported under the conditions of this test. A low level of spontaneous transformation was observed on all of the plates. Only the 3-methylcholanthrene treated plates showed a significantly higher number of transformed foci than the negative control.

It should be noted that in this and other cell culture tests, extremely low dosages of hex were used. Because hex is relatively toxic to cells in culture and test protocols normally require a high survival rate, the applicability of test results to environmental conditions is unclear. Taken together, however, the mutagenicity and carcinogenicity tests conducted by Litton (1977, 1978a) suggest that outright toxicity, rather than chronic effects, is perhaps the critical effect of hex, even at very low dosages. Extremely poor survival has also been problematic in several subchronic tests of hex in mammalian species.

A very recent study involving chronic dietary exposure of rats to hexachlorobutadiene also provides some insight into the relationship between direct toxic effects and chronic effects (i.e., carcinogenesis) in this related compound (Kociba, et al., 1977). Male and female Sprague-Dawley rats were maintained on diets supplying 20, 2.0, 0.2, or 0 mg/kg/day of hexachlorobutadiene (C-46) for up to 2 years. Rats ingesting 0.2 mg/kg/day had no discernible ill effects that could be attributed to this dose level. Ingestion of the intermediate dose level of 2.0 mg/kg/day caused some degree of toxicity, affecting primarily the kidney in which increased renal tubular epithelial hyperplasia was noted. Urinary excretion of coproporphyrin was also increased at this dose level. Ingestion of the highest dose level of 20 mg/kg/day caused a greater degree of toxicity. Effects included decreased body weight gain and length of survival, increased urinary excretion of coporporphyin, increased weights of kidneys, and renal tubular adenomas and adenocarcinomas, some of which metastasized to the lung. In this study irreversible toxicological effects, such as the developmment of neoplasms, occurred only at a dose level which caused significant tissue injury and other manifestations of toxicity. No neoplasms resulted with dose levels which caused no injury or only mild, reversible injury.

Little information is available on the metabolism of hex. Although at least four metabolites were found in the Mehendale (1977) study, at the time of this writing they had not been identified. Thus, the metabolic pathway is uncertain.

As far as structure/activity relationships are concerned, the National Cancer Institute (1977a) speculated that as a cyclopentene vinyl halide, hex potentially may be metabolized to an electrophile. In addition, hex is related to the pesticides dieldrin, heptachlor, and chlordane which have been found to induce liver tumors in mice following oral administration (National Cancer Inst., 1977b; 1977c).

Hex has recently been selected for testing in the National Cancer Institute's test program (National Cancer Inst., 1977a). The reasons given for its selection include: (1) its high potential for exposure (as an industrial intermediate used in the manufacture of pesticides, flame retardants and dyes, pharmaceuticals, resins, and germicides); (2) its suspect chemical structure; and (3) the relative lack of information on the effects of chronic exposure to this compound.

5.0 HUMAN TOXICITY

Very little information is available concerning the effects of hex exposure on humans. Unfortunately, no systematic epidemiologic investigations of the toxicity of this compound have been reported. Nevertheless, several reasonable inferences might be made on the basis of animal studies. From the early studies of Treon, et al., (1955) it is apparent that hex is an extremely potent irritant and is toxic by all these major modes of exposure; oral, dermal and inhalation. Furthermore, animal studies indicate that the relative toxicities of hex via oral and dermal exposures are remarkably similar. It is presently unknown whether oral exposure (e.g. through ingestion of hex-contaiminated drinking water) constitutes a significant source of human exposure. Oral contact does not appear to be a likely mode of occupational exposure. However, dermal and inhalation exposures to hex might be anticipated among workers engaged directly in hex manufacture or in formulation of other related pesticides where it may be present as an impurity. A recent incident in which scores of workers at a sewage treatment plant in Louisville, Kentucky, experienced a variety of toxic symptoms following the improper disposal of hex manufacturing wastes has created a great demand for information concerning the effects of hex exposure on humans. This episode is described in greater detail later in this section.

It is essential that persons having opportunities for skin contact with hex should be equipped with, and trained in the use of appropriate protective clothing and respiratory protection. The product bulletin on hex (Velsicol, 1976) states that skin contact should be avoided and persons handling hex should be outfitted with Neoprene gloves and protective goggles and face shields. Adequate protective clothing should be worn at all times.

5.1 DETECTION THRESHOLDS

According to Treon, et al., (1955) a very faint odor of hex was detectable in air by some individuals at concentrations as low as 0.15 ppm (.0017 mg/l) which was the lowest concentration employed in their experiments. At approximately twice that concentration (0.33 ppm) a very pronounced, pungent odor was present.

Treon, et al., observed that headaches developed among laboratory workers following incidental exposure to hex vapor from the respiratory chambers used for their vapor inhalation experiments. The exact concentration of hex escaping into the laboratory from the opening of the respiratory chamber is unknown, however the chamber was not opened until the contaminated air had been exhausted and the chamber flushed for some time with clean air. Thus, the ambient concentration producing headaches among the laboratory workers was well below the doasges employed in the animal experiments. Because no mention is made of any other irritative symptons, (e.g. lacrimation, etc.) it seems reasonable to speculate that the concentration of hex present was somewhere in the range between 0.15 ppm - 1.0 ppm, above the detection threshold but below the level producing acute symptoms of irritation.

Irritant effects are elicited at a vapor concentration greater than that shown to produce chronic toxicity in animals. Thus, Treon, et al., concluded that the irritant effects of hex vapors are not sufficiently pronouced to serve as a warning that a hazardous level of hex vapor is present and/or that hazardous exposure is taking place.

According to Naishstein and Lisovskaya (1965) hex may be detected by taste and smell at very low concentrations in water. The threshold level for altering the organoleptic qualities of water was placed at 0.0014 - 0.0010 mg/l by these investigators.

The toxicity of hex has some implications for standards setting. Prolonged intermittent exposure of animals to hex vapors at concentrations as low as 0.15 ppm induced slight degenerative changes in several organ systems. No overt signs of toxicity were noted at the time of exposure, however, the pattern of exposure employed in Treon, et al's (1955) low dose, chronic toxicity studies represents a reasonably close approximation to an occupational exposure pattern. (e.g. exposure to 0.15 ppm for 7 hrs. per day, 5 days per week). Although systematic observations of workers exposed chronically to known concentrations of hex are necessary to establish safe limits of human exposure, Treon, et al., concluded that, "at the very least, it would seem unwise to expose workers to even the least severe of the exposure conditions (0.15 ppm) unless or until there is some basis in terms of human experience. Men exposed to the vapor of this chlorinated hydrocarbon, even for short periods of time, should have faultless respiratory protection."

In keeping with this, Velsicol's Product Bulletin for hex (Velsicol, 1976) states that, "vapors of hex should be avoided, and adequate ventilation should always be provided when hex is handled in an enclosed area. Self-contained air masks, or full face gas masks having canisters of the 'acid gases and organic vapors' type should be available at all times for emergency use (e.g. spills)."

The present Threshold Limit Value (TLV) for industrial exposures to hex is set at 0.01 ppm as a time-weighted average over an 8 hour workday (ACGIH, 1977). This value represents about 1/15 or 7 percent of the lowest vapor concentration shown to produce chronic toxic effects in laboratory mammals (0.15 ppm; Treon, et al., 1955). The "safety factor" for hex is therefore somewhat less than for many other toxic vapors.

Similarly, Naishstein and Lisovskaya, (1965) have recommended that the maximum permissible concentration in water, based on prevention of organoleptic effects, should be placed at approximately 0.001 mg/l.

5.2 LOUISVILLE CONTAMINATION INCIDENT

The first and only documented incident of acute toxicity of hex to humans occurred at the Morris Forman Wastewater Treatment Plant (MFWTP) in southwest Louisville, Kentucky. The problem apparently began about the middle of March, 1977, when an unknown chemical, later identified as a mixture of hex and octachlorocyclopentene (C-58), began entering the Morris Forman sewage treatment facility. An exact date of initial appearance at the plant, and hence, the initial date of worker exposure is unknown. However, unusual odors became evident around March 17, 1977. There was little reaction to these odors at first, probably because unusual odors are not uncommon at sewage treatment facilities serving large industrialized areas.

The odor gradually intensified over the next two weeks. From March 25-28 an odoriferous, sticky material entered the plant and gummed the bar screens in the Screen and Grit Building of the plant. Several employees tried unsuccessfully to clean the bar screens and grit chambers with high pressure water. Subsequent steam cleaning caused a release of a blue gas into the atmosphere, producing severe irritation of the eyes, nose, throat, lungs and skin of several workers. Approximately 20 employees were referred to and treated by the local hospital emergency room. None were hospitalized (Carter, 1977; Singal, 1978).

A sample of the material from the screen and Grit Building was sent to the U.S. EPA Laboratory in Athens, Georgia, for analysis. The primary contaminants in the samples were identified as hexachlorocyclopentadiene, (hex) and octachlorocyclopentene (C-58). C-58 is a waste by-product in the manufacture of hex (See Section 2.2.2) whose toxicity is presently unknown. Upon this identification, the Morris Forman STP was evacuated and closed on March 29, 1977 and its entire flow, amounting to approximately 105 million gallons per, day was diverted directly into the Ohio River until its partial reopening in June, 1977. Analysis of a sludge sample is shown in Table 5.1.

Estimates of the extent of contamination indicate that about 60 million gallons (25,000 tons) of hex contaminated material was present at the Morris Forman plant. Of this, approximately 6 tons of hex and C-58 were thought to be present in the contaminated waste. U.S. EPA's analyses revealed hex concentrations up to 1000 ppm in the sewage water at the time of the plant closure. The route of chemical contamination was traced to one large sewer line which passed through several heavily populated areas. Wastewater in this sewer showed hex and C-58 in concentrations ranging up to 100 ppm. Samples from the sewer showed air concentrations ranging up to 400 ppb (0.4 ppm) for hex and up to 30 ppb (0.030 ppm) for C-58. Thus, it was decided to investigate the health of not only the workers at the sewage treatment plant, but also residents of the area surrounding the sewer line.

A cooperative investigation was intiated involving Region IV U.S. EPA (Surveillance and Analysis Division), Center for Disease Control

TABLE 5.1 ANALYSIS OF SLUDGE SAMPLE FROM LOUISVILLE, KENTUCKY WASTEWATER TREATMENT PLANT[a, b]

COMPOUND[c, d]	CONCENTRATION, WT. PERCENT	
Octachlorocyclopentene	9	
Hexachlorocyclopentadiene	4	
Hexachlorobenzene	0.3	
Pentachlorobenzene	0.2	
Octachloronaphthalene	0.4	(estimated)
Heptachloronaphthalene	0.2	(estimated)
Hexachloronaphthalene		(not quantitated)
Mirex	0.007	(estimated)

[a] Source: Singal (1978).

[b] Sample obtained April 2, 1972 from Screen and Grit Building, Morris Forman Wastewater Treatment Plant.

[c] Analysis was conducted by the U.S. Food and Drug Administration, Division of Chemical Technology, Chemical Industry Practices Branch.

[d] The sample was analyzed using gas chromatography interphased with mass spectroscopy for positive identification of each compound.

(Atlanta, Georgia), National Institute for Occupational Safety and Health (NIOSH), Jefferson County (Kentucky) Health Department, and the Kentucky State Health Department.

Information on both aspects of this investigation (e.g. community residents on the one hand and exposed workers on the other) is thus far unpublished, but preliminary drafts of reports were made available by Dale Morse, M.D., who headed the epidemiologic studies conducted by the Center for Disease Control (Morse, et al., 1978), and by Mitchell Singal of the Hazard Evaluation and Technical Assistance Branch of NIOSH, who reported on the follow-up investigations of workers during cleanup operations at the sewage treatment facility (Singal, 1978). Findings from these draft reports are summarized below: however, they should be regarded as preliminary.

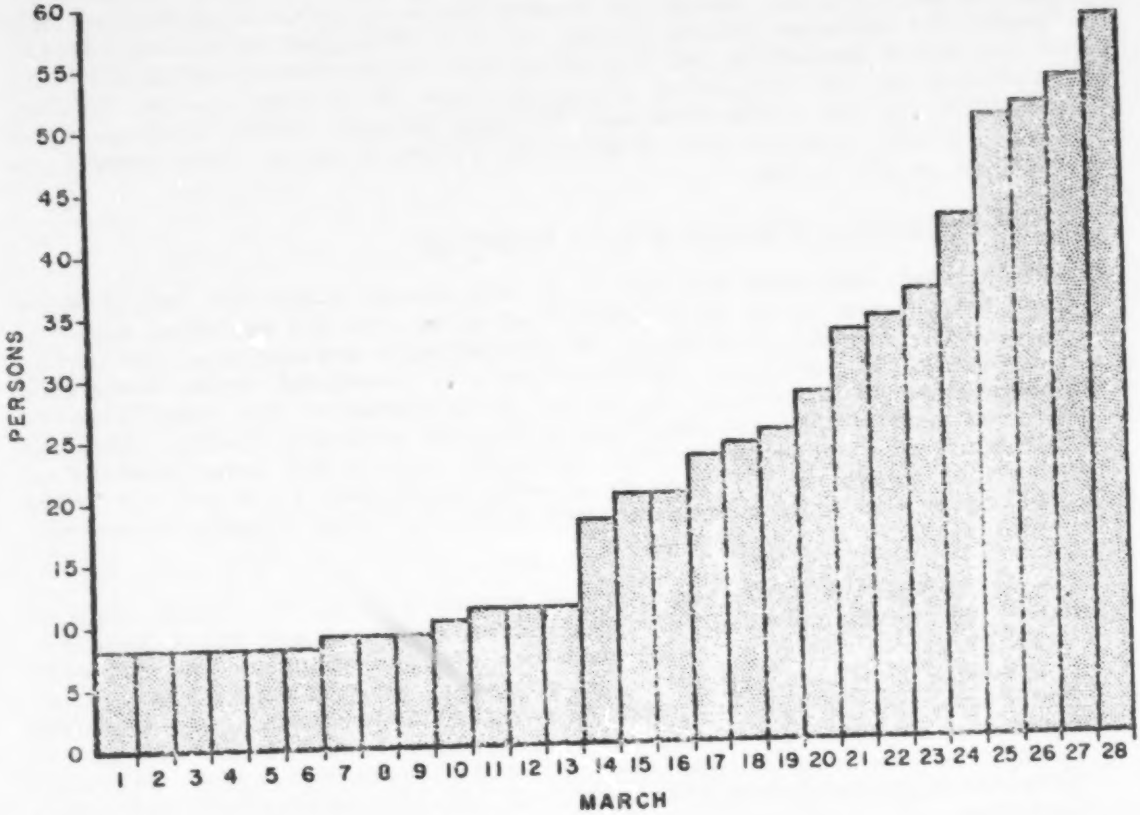
5.2.1 Plant Employee Health Effects Evaluation

All plant employees who worked at the sewage plant for two or more days from March 14-29 were identified along with all employees known to have sought medical treatment. Health effects evaluations, including questionnaires, physical examinations and blood and urine tests were performed on 42 individuals who agreed to participate. The questionnaire covered demographic information, a detailed work-area history, symptoms or history of chemical poisoning, personal habits and other sources of chemical exposure. Routine tests were performed on blood and urine specimens. Additional samples were sent to NIOSH laboratories for potential toxic chemical analyses.

Of 193 plant employees who had worked during the latter part of March, 42 persons were interviewed and provided blood and urine samples. This includes 24 of 29 (83 percent) of those workers who had been previously evaluated by local physicians, 17 of 164 of other plant employees (a 10 percent random sample) as well as 1 non-employee accidently exposed to the contaminated sludge. In addition, 104 of the remaining employees completed a mailed questionnaire. Overall, questionnaire data were obtained from 145 (75 percent) of 193 total employees.

Results of the questionnaire indicated that 75 percent of the employees detected an unusual odor at the plant sometime during March. The odor was reported as early as March 1, 1977, noticeably increased by March 14 and from then steadily increased until the plant was closed on March 29. (see Figure 5.1).

A comparison between the time of odor detection and the onset of eye irritation, the most common sympton, showed that irritation developed on the same day in 45 percent of individuals, within 1-5 days in 28 percent and after 5 days in 21 percent. Only 6 percent of employees reported onset of symptoms prior to noticing an unusual odor at the plant.



*PLANT CLOSED MARCH 29

FIGURE 5.1 Employees who noticed unusual odor at plant, by day, Louisville, Kentucky, March 1-28*, 1977
Adapted from Morse, et al.(1978).

Eye irritation, headache and throat irritation were the most common symptoms, with 59 percent, 45 percent and 27 percent of employees reporting these symptoms, respectively. Data for these and other symptoms are reported in Table 5.2. Of 41 workers physically examined, 5 had signs of eye irritation (tearing and/or redness) and 5 had signs of skin irritation. Abnormalities were found in laboratory analyses of some of the workers (e.g., LDH elevations in 27 percent and proteinuria in 15 percent of those examined). These results are inconclusive, possibly indicating a transitory abnormality or a problem with the laboratory analysis. There were no LDH or urinalysis abnormalities found on repeat tests run three weeks later by another laboratory. Also, no abnormalities were reported among individuals seen at the local hospital or by the plant physician. Thus, the validity of these laboratory test results may be questionable. Attempts to develop a technique to isolate and identify* concentrations of hex in blood and urine specimens were unsuccessful.

Employees worked primarly in one of the eight work areas shown in Table 5.3. Symptoms occurred in workers of all job categories and in all work areas. Only small differences in case rates appeared by work area although the highest attack rates occurred in workers in the primary treatment area where the level of hex was presumably highest.

Detailed work area histories on 124 individuals during the highest exposure period (Table 5.4) showed that "cases" occurred in all areas of the plant. A case was defined as an individual who reported 2 or more major symptoms (eye irritation and headaches) or 1 major symptom and 2 minor ones (sore throat, cough, chest pain, difficulty breathing, skin irritation). Attack rates were significantly higher for individuals who had been exposed to the screen and grit chamber ($p < .0001$) and to the primary settling area ($p < .02$) than for workers not exposed to these areas.

This investigation demonstrated that 64 of 145 (44 percent) of current employees questioned at the waste-water treatment plant had experienced headache and mucous membrane, skin and respiratory tract irritation after exposure to airborne hex. Highest attack rates occurred among workers in the primary treatment area where exposure was highest and ventilation poorest. In most cases symptoms were transient, but in some workers, they persisted for several days. This episode clearly demonstrates the volatility of hex and its potential for having a toxic effect on humans. Unfortunately, the long-term effects of transient exposures such as this incident are presently unknown.

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*Velsicol has recently developed a technique for the analysis of hex in human urine with a reported detectability limit of 0.3 ppb (Whitacre, 1978).

TABLE 5.2 SYMPTOMS OF 145 PLANT EMPLOYEES LOUISVILLE, KENTUCKY, MARCH, 1977[a]

Symptom	With Symptom	Percent With Symptom
Eye irritation	86	59
Headache	65	45
Throat irritation	39	27
Nausea	31	21
Skin irritation	29	20
Cough	28	19
Chest pain	28	19
Difficult breathing	23	16
Nervousness	21	14
Abdominal cramps	17	12
Decreased appetite	13	9
Decreased memory	6	4
Increased saliva	6	4

[a] Source: Morse, et al.(1978).

TABLE 5.3. ATTACK RATES IN EMPLOYEES, BY MAIN WORK AREA[a] LOUISVILLE, KENTUCKY, MARCH, 1977

Main Work Area	Number of Employees	Number Reporting Symptoms	Percentage of Employees Reporting Symptoms	Percentage of Cases of Those Reporting Symptoms
Primary treatment	19	17	89	59
Throughout plant	71	54	76	48
Vacuum filtration	19	15	79	47
Secondary aeration chamber	14	12	86	42
Administration and laboratory	30	22	73	41
Final effluent pump station	10	5	50	40
Low pressure oxidation	13	10	77	30
Incineration	17	10	59	20
Totals	193	145	75	44

[a] Source: Morse, et al(1978).

TABLE 5.4. CASE ATTACK RATES IN 124 EMPLOYEES EXPOSED TO PLANT WORK AREAS LOUISVILLE, KENTUCKY, MARCH 25-28, 1977[a]

Work Area	Number Persons Exposed to Area	Number Cases Among Exposed[b]	Attack Rate for Exposed, Percent	Attack Rate for Nonexposed, Percent	χ^2[c]	P[d]
Screen and grit	38	29	76	35	16.52	10^{-4}
Primary settling tanks	41	26	63	40	5.25	.02
Vacuum filtration	36	12	33	53	3.36	NS
Secondary treatment	39	22	56	44	1.30	NS
Sludge	32	17	53	46	.27	NS
Incineration	33	14	42	49	.24	NS
Low pressure oxidation	37	16	43	49	.19	NS
Administration building	12	6	50	47	.02	NS
Oxygen generation	18	8	44	48	.00	NS

[a] Source: Morse, et al (1978).

[b] See text for definition of a "case".

[c] Results of Chi-square test of significance of association between number of "cases" and the specific work area indicated.

[d] Significance level of the Chi-square test for the work area indicated. NS means "not significant".

5.2.2 Community Survey--

CDC workers administered a questionnaire to a systematically selected sample of residents in a 48 block area surrounding the contaminated sewer line. One home per block was surveyed by administering a questionnaire to the head of each household. In all, 212 occupants of the 48-block area were surveyed. Questions were asked concerning basic demographic data, history of unusual odors and any symptoms noted by household members within the prior two weeks.

Results of the community survey were essentially negative. Eight of the 212 persons (3.8 percent) reported noticing an uunusual odor at some time during the preceding two weeks. While some of the respondents reported symptoms compatible with hex exposure (headache 4.7 percent, burning or watering eyes 4.7 percent) No symptom occurred at greater than background rates. Symptoms not associated with hex were reported just as frequently as those possibly related to exposure. Furthermore, there was no association between symptom rates and distance from the sewer line. Subsequent air sampling failed to show a significant ambient concentration of hex in the sewer line area.

5.3 CARCINOGENICITY

Hex has recently been selected for testing in the National Cancer Institutes (NCI) test program (NCI, 1977a). The reasons given for its selection include: (1) its high potential for exposure (as an industrial intermediate); (2) its suspect chemical structure and; (3) the relative lack of information on the effects of chronic exposure to this compound.

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